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A Study of the Germicidal and Bacteriostatic Properties of Some Commonly Used Antiseptics in Combination with Astringent Substances*†

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In the history of disinfection, astringent and germicidal substances always have been somewhat inseparable from the standpoint of both use and study. There is no sharp line of distinction between the two types of agents, although astringents are generally defined as substances which are capable of condensing tissue and precipitating protein, whereas germicides are clearly substances which are capable of destroying bacteria, particularly pathogenic bacteria. Astringents do possess some antibacterial value, and they formerly were believed to be among the most effective of the disinfectants; however, this generalization is now known to be unreliable, and, with the exception of the soluble salts of mercury and silver, astringents are credited with relatively slight disinfectant value. On the other hand, combinations of astringent and purely disinfectant substances long have been used, and are still finding considerable favor in the practice of medicine. In earlier years the astringent substances were used because of their supposed disinfectant value; however, now they are generally employed because of their astringency, since a contractile action is often a desirable adjunct to a germicidal action. Nevertheless, this use gives rise to the question whether the disinfectant action of the germicide is interfered with, enhanced, or unaltered by the presence of the astringent material.

A number of recent investigations indicate that the addition of astringent substances to disinfectant preparations may have an enhancing effect upon the germicidal power of the disinfectant. Hartmann and Schelling (1) found that the effectiveness of many carbocyclic antiseptics is increased by the addition of certain inorganic acids and of tannic acid. Goedrich (2) reported an enhancing effect of acids and of acid salts upon the germicidal actions of all commonly used types of disinfectants. Goshorn, Degering and Tetrault (3) have found that benzoic acid is more effective in solutions of low *p*H value. These researches have a bearing on the problem under consideration when it is remembered that tannic acid is an astringent and that the inorganic astringent salts hydrolyze in solution to give solutions of low *p*H.

The present investigation was begun in an attempt to determine what effects, if any, the addition of astringent substances may have upon the germicidal and bacteriostatic

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actions of some commonly used germicides. It was intended that the experiments should be sufficiently well controlled so that some information might be gained concerning the cause of any observed effects.

EXPERIMENTAL

Since it was intended that this investigation should deal with preparations which are in common use, the antiseptics were chosen with two points in mind, common usage and compatability with the astringent substances; but the investigation was by no means all-inclusive. Antiseptics used were phenol, hexylresorcinol,[1] merthiolate and metaphen (the latter two in the form of the tincture). The astringents were various salts of aluminum, copper, iron, lead, mercury, silver and zinc; and tannic acid. Tannic acid was chosen as representative of the class of vegetable astringents, whereas the inorganic astringents were chosen with the guidance of Sollmann (4). No representatives of the class of miscellaneous astringents were used, since it was believed that their mechanism of action is so complex that they could not be expected to have a typically astringent effect upon simple organisms.

Determination of Disinfectant Powers of the Combinations.—Solutions for the tests were made by adding sufficient amounts of the inorganic astringents to each of the antiseptic solutions to make the solution one-tenth molar with respect to the astringent. When tannic acid was used, a sufficient amount of this substance was added to make the solution contain either 5 or 10% (weight in volume) of tannic acid. The antiseptic solutions to which these astringents were added were preparations of the antiseptic substances in the form in which they are commonly used. Because of the tendency to hydrolysis of the astringent salts, many of the solutions were not clear when prepared in this way, but contained a cloudy precipitate of the hydrated oxide of the metal. When this condition existed, sufficient acid was added to render the solution clear. Tenth-normal hydrochloric acid was employed except when it would have been incompatible with the astringent present. The *p*H value of the antiseptic-astringent combinations was then determined by using the Hellige *p*H meter. The potentiometer was checked against a standard buffer both before and after taking each reading.

Solutions were now made containing one-tenth molar amounts of the astringent salts (10% of tannic acid) in the same solvent as that which existed in the antiseptic-astringent combination, and the *p*H value of these solutions was adjusted to the same value as that of the corresponding combination.

Sufficient acid was added to some of the pure antiseptic solution to adjust its *p*H value to that of each of the pairs of solutions described. Thus 3 solutions were prepared for each combination used.

Disinfectant properties of all these solutions were determined by using the F. D. A. method of antiseptic testing (5). All experimental results were rechecked at least once before being accepted. In accordance with the suggestion of Birkhaug (6), a loopful of 24-hr. culture was introduced into each tube of broth which was negative at the time of the 48-hr. reading. This precaution prevented any error which might have resulted from negative readings caused by the inhibiting power of antiseptic carried over from the seeding tube to the broth tube.

Results of the experiments conducted in this manner are recorded in Tables I to VI, inclusive. The key to the columns in the tables follows:

Column I—Actual dilution of combination which kills in 10 min. but not in 5 min.
Column II—Concentration of antiseptic present in the dilution represented in Column I.
Column III—Concentration of astringent present as in II.
Column IV—Concentration of antiseptic which ordinarily kills in 10 min. but not in 5 min.
Column V—Concentration of astringent which ordinarily kills in 10 min. but not in 5 min.
Column VI—Percentage increase in apparent germicidal efficiency of the antiseptic. These are calculated to the nearest 5%.

[1] Supplied by the Sharp and Dohme Laboratories, Philadelphia, Pa.

TABLE I.—GERMICIDAL EFFICIENCY OF PHENOL PLUS ASTRINGENT SUBSTANCES

Combinations	*p*H	I	II	III	IV	V	VI
5% Phenol + *M*/10 $AlCl_3$	3.30	1:7½	1:150	*M*/75	1:90	...[a]	70%
5% Phenol + *M*/10 $Al_2(SO_4)_3$	3.50	1:7	1:140	*M*/70	1:90	...	50%
5% Phenol + *M*/10 K Alum	3.50	1:6	1:120	*M*/60	1:90	...	35%
5% Phenol + *M*/10 $CuCl_2$	3.60	1:22½	1:450	*M*/225	1:90	*M*/35	400%
5% Phenol + *M*/10 $CuSO_4$	3.60	1:11½	1:225	*M*/115	1:90	*M*/10	150%
5% Phenol + *M*/10 $FeCl_3$	2.20	1:12½	1:250	*M*/125	1:120	*M*/25	110%
5% Phenol + *M*/10 $Fe_2(SO_4)_3$	2.30	1:37½	1:750	*M*/375	1:120	*M*/150	525%
5% Phenol + *M*/10 $PbAc_2$	5.00	1:7½	1:150	*M*/75	1:90	...	70%
5% Phenol + *M*/10 $ZnAc_2$	6.00	1:4½	1:90	*M*/45	1:90	...	0%
5% Phenol + *M*/10 $ZnCl_2$	5.35	1:8	1:160	*M*/80	1:90	...	75%
5% Phenol + *M*/10 $ZnSO_4$	4.90	1:6½	1:130	*M*/65	1:90	...	40%
5% Phenol + *M*/10 Zn phenolsulfonate	6.40	1:6	1:120	*M*/60	1:90	...	35%
5% Phenol + 10% Tannic Acid	5.00	1:9½	1:190	1:95	1:90	1:20	110%

[a] Indicates that the astringent will not kill the test organism in 10 min. in the concentration used in the combination.

TABLE II.—GERMICIDAL EFFICIENCY OF SOLUTION S.T. 37 PLUS ASTRINGENT SUBSTANCES

Organism: *Eberthella typhosa*

Combinations	pH	I	II	III	IV	V	VI
S.T. 37 + *M*/10 $AlCl_3$	3.00	1:10	1:10,000	*M*/100	1:7500	...[a]	25%
S.T. 37 + *M*/10 $Al_2(SO_4)_3$	3.00	1:10	1:10,000	*M*/100	1:7500	...	25%
S.T. 37 + *M*/10 $Al(NO_3)_3$	2.90	1:10	1:10,000	*M*/100	1:8000	...	20%
S.T. 37 + *M*/10 K Alum	3.10	1:10	1:10,000	*M*/100	1:7500	...	25%
S.T. 37 + *M*/10 $CuCl_2$	3.55	1:35	1:35,000	*M*/350	1:7000	*M*/80	400%
S.T. 37 + *M*/10 $CuSO_4$	3.85	1:30	1:30,000	*M*/300	1:7000	*M*/30	330%
S.T. 37 + *M*/10 $Cu(NO_3)_2$	3.90	1:30	1:30,000	*M*/300	1:7000	*M*/30	330%
S.T. 37 + *M*/10 $PbAc_2$	5.65	1:15	1:15,000	*M*/150	1:6500	...	130%
S.T. 37 + *M*/10 $Pb(NO_3)_2$	3.80	1:15	1:15,000	*M*/150	1:6500	...	130%
S.T. 37 + *M*/10 $Fe_2(SO_4)_3$	1.30	1:35	1:35,000	*M*/350	1:20,000	*M*/150	75%
S.T. 37 + *M*/10 $Fe(NO_3)_3$	1.50	1:15	1:15,000	*M*/150	1:17,500	*M*/40	15%
S.T. 37 + *M*/10 $ZnAc_2$	5.65	1:10	1:10,000	*M*/100	1:6500	...	55%
S.T. 37 + *M*/10 $ZnCl_2$	5.60	1:11	1:11,000	*M*/110	1:6500	...	70%
S.T. 37 + *M*/10 $ZnSO_4$	5.65	1:8	1:8000	*M*/80	1:6500	...	25%
S.T. 37 + *M*/10 Zn Phenolsulfonate	5.60	1:9	1:9000	*M*/90	1:6500	...	40%
S.T. 37 + *M*/10 $AgNO_3$		1:40	1:40,000	*M*/400	1:6500	*M*/400	0%[b]
S.T. 37 + *M*/100 $HgCl_2$	3.00	1:450	1:450,000	*M*/45,000	1:6500	*M*/45,000	0%[b]
S.T. 37 + 10% Tannic Acid	3.50	1:20	1:20,000	1:200	1:7000	...	200%

[a] Indicates that the astringent will not kill the test organism in 10 min. in the concentration used in the combination.
[b] No increase is reported since the astringent salts are as powerful as the combinations.

TABLE III.—GERMICIDAL EFFICIENCY OF TINCTURES OF MERTHIOLATE AND OF METAPHEN PLUS ASTRINGENT SUBSTANCES

Organism: *Eberthella typhosa*

Combinations	pH	I	II	III	IV	V	VI
Tincture of Merthiolate							
Tr. Merth. + *M*/10 $AlCl_3$	3.50	1:45	1:45,000	*M*/450	1:20,000	*M*/30	125%
Tr. Merth. + *M*/10 $Cu(NO_3)_2$	2.20	1:35	1:35,000	*M*/350	1:25,000	*M*/100	40%
Tr. Merth. + *M*/10 $FeCl_3$	1.85	1:35	1:35,000	*M*/350	1:25,000	*M*/90	40%
Tr. Merth. + *M*/10 $PbAc_2$	4.20	1:40	1:40,000	*M*/400	1:8000	*M*/20	400%
Tr. Merth. + *M*/10 $ZnCl_2$	4.00	1:20	1:20,000	*M*/200	1:8000	*M*/30	50%
Tr. Merth. + 5% Tannic Acid	6.00	1:10	1:10,000	1:200	1:6000	1:40	65%
Diluted Tincture of Metaphen[a]							
Dil. Tr. Met. + *M*/10 $AlCl_3$	3.50	1:200	1:200,000	*M*/2000	1:100,000	*M*/30	100%
Dil. Tr. Met. + *M*/10 $CuCl_2$	3.60	1:225	1:225,000	*M*/2250	1:100,000	*M*/100	125%
Dil. Tr. Met. + *M*/10 $FeCl_3$	1.60	1:225	1:225,000	*M*/2250	1:200,000	*M*/90	10%
Dil. Tr. Met. + *M*/10 $PbAc_2$	5.00	1:300	1:300,000	*M*/3000	1:90,000	*M*/20	235%
Dil. Tr. Met. + *M*/10 $ZnCl_2$	5.00	1:225	1:225,000	*M*/2250	1:90,000	*M*/30	150%
Dil. Tr. Met. + 5% Tannic Acid	6.00	1:200	1:200,000	1:4000	1:40,000	1:40	400%

[a] Made by diluting regular Tincture of Metaphen (1:200) with the usual solvent until it contains 1:1000 of metaphen.

TABLE IV.—GERMICIDAL EFFICIENCY OF PHENOL PLUS ASTRINGENT SUBSTANCES

Organism: *Staphylococcus aureus*

Combinations	pH	I	II	III	IV	V	VI
5% Phenol + *M*/10 $AlCl_3$	3.30	1:4½	1:90	*M*/45	1:80	...[a]	10%
5% Phenol + *M*/10 $Al_2(SO_4)_3$	3.50	1:7	1:140	*M*/70	1:80	...	75%
5% Phenol + *M*/10 K Alum	3.50	1:4	1:80	*M*/40	1:80	...	0%
5% Phenol + *M*/10 $CuCl_2$	3.60	1:7½	1:150	*M*/75	1:80	*M*/10	90%
5% Phenol + *M*/10 $CuSO_4$	3.60	1:5½	1:110	*M*/55	1:80	...	40%
5% Phenol + *M*/10 $FeCl_3$	2.20	1:6½	1:130	*M*/65	1:90	...	45%
5% Phenol + *M*/10 $Fe_2(SO_4)_3$	2.30	1:8	1:160	*M*/80	1:90	*M*/10	80%
5% Phenol + *M*/10 $PbAc_2$	5.00	1:4	1:80	*M*/40	1:80	...	0%
5% Phenol + *M*/10 $ZnAc_2$	6.00	1:4	1:80	*M*/40	1:80	...	0%
5% Phenol + *M*/10 $ZnCl_2$	5.35	1:4½	1:90	*M*/45	1:80	...	10%
5% Phenol + *M*/10 $ZnSO_4$	4.90	1:4½	1:90	*M*/45	1:80	...	10%
5% Phenol + *M*/10 Zn Phenolsulfonate	6.40	1:4½	1:90	*M*/45	1:80	...	10%
5% Phenol + 10% Tannic Acid	3.50	1:7	1:170	1:70	1:80	...	75%

[a] Indicates that the astringent is unable to kill in a concentration of 1/10 molar (10% for tannic acid) in 10 min.

TABLE V.—GERMICIDAL EFFICIENCY OF SOLUTION S.T. 37 PLUS ASTRINGENT SUBSTANCES

Organism: *Staphylococcus aureus*

Combination	ϕH	I	I	III	IV	V	VI
S.T. 37 + *M*/10 $AlCl_3$	3.20	1:7½	1:7500	*M*/75	1:6250	...[a]	20%
S.T. 37 + *M*/10 $Al_2(SO_4)_3$	3.23	1:6½	1:6500	*M*/65	1:6250	...	5%
S.T. 37 + *M*/10 $Al(NO_3)_3$	3.15	1:7½	1:7500	*M*/75	1:6250	...	20%
S.T. 37 + *M*/10 K Alum	3.10	1:8	1:8000	*M*/80	1:6250	...	30%
S.T. 37 + *M*/10 $CuCl_2$	3.55	1:12½	1:12,500	*M*/125	1:6250	...	100%
S.T. 37 + *M*/10 $CuSO_4$	3.85	1:10	1:10,000	*M*/100	1:6250	...	60%
S.T. 37 + *M*/10 $Cu(NO_3)_2$	3.75	1:10	1:10,000	*M*/100	1:6250	...	60%
S.T. 37 + *M*/10 $FeCl_3$	1.65	1:12½	1:12,500	*M*/125	1:9000	*M*/10	40%
S.T. 37 + *M*/10 $Fe_2(SO_4)_3$	1.50	1:10	1:10,000	*M*/100	1:9000	*M*/15	10%
S.T. 37 + *M*/10 $Fe(NO_3)_3$	1.50	1:22½	1:22,500	*M*/225	1:9000	*M*/10	150%
S.T. 37 + *M*/10 $HgCl_2$	5.00	1:500	1:500,000	*M*/5000	1:6250	*M*/5000	0%[b]
S.T. 37 + *M*/10 $PbAc_2$	5.65	1:7½	1:7500	*M*/75	1:6000	...	25%
S.T. 37 + *M*/10 $Pb(NO_3)_2$	3.80	1:8	1:8000	*M*/80	1:6250	...	20%
S.T. 37 + *M*/10 $ZnAc_2$	5.65	1:6½	1:6500	*M*/65	1:6000	...	10%
S.T. 37 + *M*/10 $ZnCl_2$	5.60	1:8½	1:8500	*M*/85	1:6000	...	40%
S.T. 37 + *M*/10 $ZnSO_4$	5.65	1:6½	1:6500	*M*/65	1:6000	...	10%
S.T. 37 + *M*/10 Zn Phenolsulfonate	5.60	1:7	1:7000	*M*/70	1:6000	...	15%
S.T. 37 + 10% Tannic Acid	4.00	1:9½	1:9500	1:95	1:6250	...	50%

[a] Indicates that the astringent is unable to kill in a concentration of 1/10 molar (10% for tannic acid) in 10 min.
[b] No increase is reported since the astringent salt is as powerful as the combi nation.

TABLE VI.—GERMICIDAL EFFICIENCY OF TINCTURES OF MERTHIOLATE AND OF METAPHEN PLUS ASTRINGENT SUBSTANCES

Organism: *Staphylococcus aureus*

Combinations	ϕH	I	II	III	IV	V	VI
Tincture of Merthiolate							
Tr. Merth. + *M*/10 $AlCl_3$	3.50	1:12½	1:12,500	*M*/125	1:7500	...	65%
Tr. Merth. + *M*/10 $Cu(NO_3)_2$	2.20	1:12½	1:12,500	*M*/125	1:10,000	*M*/30	25%
Tr. Merth. + *M*/10 $FeCl_3$	1.85	1:15	1:15,000	*M*/150	1:10,000	*M*/30	50%
Tr. Merth. + *M*/10 $PbAc_2$	4.20	1:7½	1:7500	*M*/75	1:5000	...	50%
Tr. Merth. + *M*/10 $ZnCl_2$	4.00	1:12½	1:12,500	*M*/125	1:5000	...	150%
Tr. Merth. + 5% Tannic Acid	5.00	1:10	1:10,000	1:200	1:4000	1:10	150%
Tincture of Metaphen							
Tr. Met. + *M*/10 $AlCl_3$	3.50	1:20	1:4000	*M*/200	1:1600	...	150%
Tr. Met. + *M*/10 $CuCl_2$	3.50	1:20	1:4000	*M*/200	1:1600	*M*/35	150%
Tr. Met. + *M*/10 $FeCl_3$	1.60	1:45	1:9000	*M*/450	1:4500	*M*/30	100%
Tr. Met. + *M*/10 $PbAc_2$	5.00	1:10	1:2000	*M*/20	1:1600	...	20%
Tr. Met. + *M*/10 $ZnCl_2$	5.00	1:25	1:5000	*M*/250	1:1600	...	210%
Tr. Met. + 10% Tannic Acid	5.00	1:12½	1:2500	1:125	1:1600	1:10	55%

Discussion.—In the foregoing series of tables, the increase in germicidal efficiency brought about by a combination of astringents with antiseptic substances is reported as a percentage increase in the apparent disinfectant strength of the antiseptic. It has not been definitely established that the germicidal strength of the antiseptic (as opposed to that of the astringent) is increased; rather, this is merely an arbitrary method which has been used to emphasize the fact that the combinations are more efficient than the individual ingredients in the same concentrations.

Table I, for example, shows the germicidal efficiency of phenol solutions containing various astringent substances against the typhoid organism. It will be observed that for every combination except that of phenol with zinc acetate, the potency of the combination is considerably greater than that of either of the ingredients alone. For example, a combination of 5% of phenol with tenth-molar aluminum chloride will destroy the typhoid organism in 10 min. but not in 5 min. in a dilution of 1 in 7½. This dilution contains phenol in a concentration of 1 in 150 and aluminum chloride in a concentration of 1/75 molar. Phenol alone, however, must be present in a concentration of 1 in 90 in order to destroy the organism in the same time interval and under the same conditions, whereas aluminum chloride is not able to destroy the organism even in 10 min. in a concentration of one-tenth molar. The increase in the apparent germicidal strength of the phenol, then, is around 70%. This instance is only one of many examples which might be selected from the tables.

It was observed that the increase in germicidal efficiency occasioned by the addition of the astringents to phenol parallels, in general, the disinfectant strengths of the astringents alone (6). This seems to be an indication that the increase is in some way an intrinsic property of the astringent substances. It is interesting to note that combinations of phenol with iron salts were quite effective even though the

solutions develop the purplish-brown color which characterized the iron test for phenol. These solutions were not stable for long, however, and the germicidal ability was found to remain high only as long as the solutions remained free from precipitate.

Table II shows the experimental results obtained when Solution S.T. 37 containing astringent substances was tested by the F. D. A. method. A more complete series of salts was used with this solution. Here again it seems evident that a combination of substances of astringent nature with antiseptics is frequently followed by an increased germicidal efficiency.

In conducting the experiments reported in Table III, considerable difficulty was encountered. The tinctures of metaphen and of merthiolate are alcoholic solutions, hence only alcohol-soluble astringents could be used in making these combinations. Since the tinctures are alkalinized solutions in which the astringent salts would have been quickly thrown down as hydroxides, it was necessary to add enough acid to bring the tincture to neutrality before the salt was added. For this purpose concentrated hydrochloric acid was used in instances where it was compatible. Usually 1 or 2 drops of the concentrated acid were required. After the astringent salt had been added, if more acid was needed to render the solution clear, tenth-normal hydrochloric acid was used except when it would have been incompatible. Solutions of the astringent salts in acid Tincture of Merthiolate were found to be surprisingly clear and stable, but some of them did not behave very well when diluted. Solutions having a *p*H value greater than 4.5 and less than 7.0 deposited a rather flocculent precipitate. Tincture of Metaphen solutions at approximately the same *p*H range behaved in a similar manner.

One might anticipate that merthiolate, being a sodium salt, would be converted to the corresponding acid by inorganic acids and be precipitated. This seems to be only partially true, since Tincture of Merthiolate at a *p*H value of 3.5 seems to have about the same germicidal value as the ordinary Tincture of Merthiolate which has a *p*H value of 10.5. On the other hand, at *p*H values near neutrality the germicidal power of Tincture of Merthiolate is greatly reduced.

Tables IV, V and VI present similar data as the preceding tables, except that the test organism was changed. In these tables are presented the results obtained from the investigation of the disinfectant powers of the antiseptic-astringent combinations when *Staphylococcus aureus* was used as the test organism. The culture of *Staphylococcus aureus* used had a resistance to phenol of 1:80 at 20° C. This resistance is slightly weaker than the official culture; however, no phenol coefficients are reported, and the results included in this thesis are entirely comparative.

With these latter tests, as previously, the germicidal efficiency of the antiseptics seems to be improved by the addition of the astringent substances. It will be observed, however, that the increase in disinfectant power is much less noticeable when the gram-positive organism is used than when the typhoid organism was employed. It has been observed in previous research (7) that *Staphylococcus aureus* is more resistant to inorganic salts than is *Eberthella typhosa*. This condition seems to be further confirmation of the belief that the increase in germicidal effect is in some way more closely associated with the inorganic salts than it is with the antiseptics.

The one instance in which a serious disagreement with previous literature was noted was in the study of the effect upon *S. aureus* of combinations of Tincture of Metaphen with the astringents. Preparations containing metaphen were found to be relatively ineffective against staphylococci. This condition may result from the fact that the preparations used had *p*H values which are unfavorable to metaphen; however, the effectiveness of the compound against *Eberthella typhosa* does not seem to be appreciably less in acid solution. Furthermore, in testing two different samples of the regular alkalinized tincture purchased in the open market, we did not find them to be as effective against staphylococci as previous literature (8) would indicate.

Determination of Inhibitory Action against the Colon Organism.—The method used in this part of the experimental work was devised to bring out any effects which the addition of astringents might have upon the inhibitory action of antiseptics. The culture used was a mixed culture prepared by combining four strains of lactose-fermenting organisms of the intestinal variety. The culture was maintained in lactose broth, one standard loopful being transferred into a fresh tube of broth every 24 hrs.

In carrying out the experiments, amounts of antiseptic sufficient to give varying concentrations were added to lactose broth contained in small test tubes equipped with inner tubes. In a similar manner, tubes containing varying concentrations of the astringents in lactose broth were prepared. Each tube was then inoculated with one standard loopful of a 24-hr. culture of the test organism. Gas production was read at 12, 24, 48 and 72-hr. intervals following the inoculations and the contents of the tubes were inspected for growth cloudiness whenever possible. It was not always feasible to determine the presence or absence of growth by the corresponding cloudiness or clearness of the broth, for some of the astringents produced considerable cloudiness by their action upon the broth. In such a case only the presence or absence of gas could be taken as the criterion of growth.

Broth tubes were next prepared containing the antiseptics in concentrations which had been found to be incapable of inhibiting growth for 48 hrs. plus the astringents in concentrations which had been shown to be incapable of inhibiting growth for 48 hrs. It was originally intended that the concentration of astringent used should be one capable of inhibiting growth for 24 but not for 48 hrs.; however, in at-

tempting to avoid cloudiness caused by precipitation of proteins from the broth, lower concentrations than this were often used. These tubes were inoculated and observed for gas production and cloudiness (when possible) as before. Only the more active of the astringents were used in carrying out these experiments. The chlorides of aluminum, copper, iron, mercury, and zinc, lead acetate, silver nitrate, and tannic acid were used.

Results of this study are presented in condensed form in Tables VII to X. Each series of figures represents the quantity of gas produced in the particular tube in 12, 24, 48 and 72 hrs., respectively.

Discussion.—In order to grasp the significance of these data, one must study the tables very closely. In Table IX, for example, 1:10,000 hexylresorcinol was unable to inhibit gas production for 48 hrs., as was *M*/500 aluminum chloride. But 1:10,000 hexylresorcinol plus *M*/500 aluminum chloride was capable of inhibiting gas production for the entire period of 72 hrs. Likewise, 1:2,500,000 merthiolate was unable to inhibit gas production for 48 hrs., and *M*/1000 $ZnCl_2$ could not inhibit gas production for even 24 hrs., but a combination of them was able to inhibit for 72 hrs. Many similar examples of apparently synergistic effects may be found in the tables.

While it must be admitted that the absence of gas is not a positive indication of the absence of growth, there are several reasons for believing that for this particular organism in lactose broth it is a very strong indication of negative growth. It seems very improbable that when neither the astringent nor the antiseptic was capable of inhibiting either growth or gas production, a combination of the two should be capable of inhibiting only the function of gas production and not the growth function.

Though the growth-inhibiting powers of all of the antiseptics were increased by combination with some of the astringents in certain concentrations, the enhancement very obviously depends upon both the nature and the concentration of both antiseptic and astringent. Lead acetate appears to have little effect upon the inhibiting power of phenol and hexylresorcinol, whereas it causes an enhancement of the inhibiting power of merthiolate and of metaphen. On the other hand, aluminum chloride enhances the inhibiting value of all of the antiseptics in some concentrations.

When the concentration of the astringent is reduced to a certain value, it seems to have little effect upon the inhibiting power of an antiseptic to which it has been added.

TABLE VII.—SHOWING THE EFFECT OF THE ADDITION OF ASTRINGENTS ON THE ABILITY OF HEXYLRESORCINOL TO INHIBIT GAS PRODUCTION BY *Escherichia coli*

Astringent Added		Astringent Alone	Concentration of Hexylresorcinol 1:10,000	1:25,000	1:50,000
Aluminum Chloride	Control		0 Tr 5 10	0 Tr 10 15	10 30 50 50
	M/500	0 Tr 5 20	0 0 0 0	0 0 0 25	0 0 15 30
	M/1000	Tr 25 30 35	0 0 0 0	5 25 60 60	0 25 30 40
	M/5000	..... [a]	0 0 5 10	.....	.....
Cupric Chloride	Control		0 Tr 5 10	0 Tr 10 15	10 30 50 50
	M/100	10 35 75 90	0 0 0 0	0 0 0 0	0 0 0 0
	M/500	10 25 45 50	0 0 0 0	0 0 0 0	10 20 40 50
Ferric Chloride	Control		Tr 5 35 60	5 10 30 45	15 40 65 70
	M/50	0 0 20 20	0 0 0 0	0 0 0 0	0 5 30 45
	M/100	5 15 30 30	0 0 0 0	Tr 10 35 45	0 10 35 45
	M/500	10 25 40 45	0 5 25 35	Tr 20 45 55	.....
Lead Acetate	Control		0 5 10 15	10 30 60 70	20 35 75 80
	M/50	0 5 15 30	0 0 0 Tr	0 10 40 50	0 5 35 40
	M/100	5 10 40 40	0 Tr 25 100	5 40 85 85	10 30 85 85
	M/500	10 15 40 50	Tr 10 45 55	0 15 50 85	20 40 75 85
Silver Nitrate	Control		Tr 5 15 30	5 20 35 45	20 40 60 70
	M/10,000	0 Tr 15 20	0 0 0 0	0 0 0 0	0 0 0 0
	M/25,000	Tr 40 80 90	0 0 0 0	0 0 10 35	0 Tr 20 40
	M/50,000	35 60 75 90	0 0 Tr 20	5 20 35 45	.....
Zinc Chloride	Control		0 Tr 5 10	10 30 50 55	10 30 50 55
	M/1000	0 5 20 30	0 0 0 0	0 0 0 0	0 0 0 0
	M/2500	0 10 20 30	0 0 0 0	0 0 0 Tr	5 15 40 50
	M/5000	Tr 10 25 40	0 0 0 0	0 Tr 15 35	.....
Tannic Acid	Control		0 Tr 10 10	10 30 50 55	10 30 50 55
	1:500	0 0 10 40	0 0 0 0	0 0 0 0	0 0 10 20
	1:1000	Tr 10 40 60	0 0 0 0	0 0 Tr 15	0 0 15 30
	1:5000	10 20 40 60	0 0 15 20	0 Tr 30 40	0 15 60 60

[a] Indicates no tubes used with these concentrations. Figures show gas production at 12, 24, 48 and 72 hrs.

TABLE VIII.—SHOWING THE EFFECT OF THE ADDITION OF ASTRINGENTS ON THE ABILITY OF MERTHIOLATE TO INHIBIT GAS PRODUCTION BY *Escherichia coli*

Astringent Added		Astringent Alone	Concentration of Merthiolate 1:2,500,000	1:5,000,000
Aluminum Chloride	Control		0 0 5 10	0 Tr 15 20
	M/500	0 Tr 5 20	0 0 0 0	0 0 0 0
	M/1000	Tr 25 30 35	0 0 0 Tr	0 0 5 5
	M/5000	..... [a]	0 0 0 0	0 0 5 5
Cupric Chloride	Control		5 10 25 40	Tr 15 35 50
	M/100	10 35 75 90	0 0 0 0	0 0 0 0
	M/500	10 25 45 50	0 10 15 20	0 0 10 15
Ferric Chloride	Control		0 0 5 10	0 Tr 15 20
	M/50	0 0 20 20	0 0 0 0	0 0 Tr 5
	M/100	5 15 30 30	0 0 0 0	0 0 Tr Tr
	M/500	10 25 40 45	0 0 Tr Tr	0 5 10 15
Lead Acetate	Control		0 0 5 10	0 Tr 15 20
	M/50	0 5 15 30	0 0 0 0	0 0 0 0
	M/100	5 10 40 40	0 0 Tr Tr	0 0 Tr 5
	M/500	10 15 40 50	0 Tr 5 10	0 Tr 10 30
Silver Nitrate	Control		Tr Tr 5 10	5 10 15 35
	M/10,000	0 Tr 15 20	0 0 0 0	0 0 0 0
	M/25,000	Tr 40 80 90	0 0 0 0	0 Tr 5 10
	M/50,000	35 60 75 90	0 0 0 0	0 Tr 5 10
Zinc Chloride	Control		0 0 5 10	0 Tr 5 10
	M/1000	0 5 25 30	0 0 0 0	0 0 0 0
	M/5000	Tr 10 25 40	0 0 0 0	0 0 0 15
Tannic Acid	Control		0 0 5 10	0 Tr 15 20
	1:500	0 0 10 40	0 0 0 0	0 0 0 0
	1:1000	Tr 10 40 60	0 0 0 0	0 0 0 0
	1:5000	10 20 40 60	0 0 0 0	0 Tr 15 20

[a] Indicates no tubes were used with these concentrations. Figures show gas production in 12, 24, 48 and 72 hrs.

TABLE IX.—SHOWING THE EFFECT OF THE ADDITION OF ASTRINGENTS ON THE ABILITY OF METAPHEN TO INHIBIT GAS PRODUCTION BY *Escherichia coli*

Astringent Added		Astringent Alone	Concentration of Metaphen 1:500,000	1:750,000	1:1,000,000
Aluminum Chloride	Control		0 10 25 25	0 10 20 40	0 20 50 60
	M/500	0 Tr 20 20	0 0 0 0	0 0 0 0	0 5 20 25
	M/1000	Tr 25 30 35	0 0 0 0	0 5 10 10	0 5 15 25
	M/5000	..... [a]	0 0 5 10	.....	.....
Cupric Chloride	Control		0 Tr 5 10	Tr 5 10 10	10 15 25 40
	M/100	10 35 75 90	0 0 0 0	0 0 0 Tr	Tr Tr 5 5
	M/500	10 25 45 50	0 0 0 0	0 Tr 5 5	0 0 0 Tr
	M/1000	15 25 40 45	Tr Tr 5 5	Tr 10 20 25	10 20 50 75
Ferric Chloride	Control		0 10 25 25	Tr 10 25 30	Tr 20 50 60
	M/50	0 0 20 20	0 0 0 0	0 0 0 Tr	0 10 35 40
	M/100	5 15 30 30	0 0 10 20	Tr 10 20 25	0 10 15 20
	M/500	10 25 40 45	0 5 30 35	Tr 5 10 25	0 10 35 40
Lead Acetate	Control		Tr 5 5 5	10 20 40 40	5 10 30 40
	M/50	0 5 15 30	0 0 0 0	0 0 0 0	0 0 0 0
	M/100	5 10 40 40	0 Tr 5 10	Tr 5 20 50	5 10 15 40
	M/500	10 15 40 50	0 5 20 60	15 30 50 60	.....
Silver Nitrate	Control		0 0 5 10	0 0 5 10	5 20 40 50
	M/10,000	0 Tr 15 20	0 0 0 0	0 0 0 Tr	0 0 0 0
	M/25,000	Tr 40 80 90	0 0 Tr 10	0 0 0 Tr	0 0 Tr 5
	M/50,000	25 60 75 90	0 0 0 Tr	0 0 0 0	.....
Zinc Chloride	Control		0 10 25 25		0 20 50 60
	M/1000	0 5 25 30	0 0 0 0		0 0 0 0
	M/5000	Tr 10 25 40	0 0 30 50		0 15 40 40
Tannic Acid	Control		0 Tr 5 10	Tr 5 10 10	10 15 25 40
	1:500	0 0 10 40	0 0 0 0	0 0 0 0	0 0 0 0
	1:1000	Tr 10 40 60	0 0 0 Tr	0 0 0 Tr	0 Tr 15 25
	1:5000	10 20 40 60	5 20 25 35	0 Tr Tr Tr	.....

[a] Indicates no tubes were used with these concentrations. Figures show gas production in 12, 24, 48 and 72 hrs.

TABLE X.—SHOWING THE EFFECT OF THE ADDITION OF ASTRINGENTS ON THE ABILITY OF PHENOL TO INHIBIT GAS PRODUCTION BY *Escherichia coli*

Astringent Added		Astringent Alone	Concentration of Phenol 1:750	1:1000	1:1500
Aluminum Chloride	Control			0 5 15 20	5 30 30 40
	M/500	0 Tr 5 20		0 0 5 5	0 Tr 10 15
	M/1000	Tr 25 30 35		0 5 10 15	5 20 30 30
Cupric Chloride	Control		0 Tr 10 15	0 5 15 20	5 30 30 40
	M/100	10 35 75 90	0 0 0 0	0 0 0 0	0 0 Tr 15
	M/500	10 25 45 50	0 0 10 15	0 0 20 30	0 0 Tr 25
Ferric Chloride	Control		0 Tr 10 15	0 10 15 20	5 30 30 40
	M/50	0 0 20 20	0 0 Tr 10	0 0 Tr Tr	0 0 0 0
	M/100	5 15 30 30	0 5 35 40	0 10 35 40	0 5 25 30
	M/500	10 25 40 45	0 5 20 30	0 10 35 40	..... [a]
Lead Acetate	Control		10 15 15 15	10 15 15 20	Tr 10 35 40
	M/50	0 5 15 30	0 0 15 25	0 0 15 30	Tr 10 30 30
	M/100	5 10 40 40	0 0 10 15	0 10 40 40	5 10 20 20
	M/500	10 15 40 50	0 0 15 30	.....	.....
Silver Nitrate	Control		0 5 15 25	15 20 20 20	20 40 50 60
	M/10,000	0 Tr 15 20	0 0 5 15	0 0 10 25	Tr 20 60 75
	M/25,000	Tr 40 80 90	10 15 20 25	Tr 5 20 25	Tr 20 40 40
	M/50,000	35 60 75 90	10 15 20 25	5 20 25 25	.....
Zinc Chloride	Control		10 10 20 20	20 25 30 35	20 25 30 40
	M/1000	0 5 20 30	0 0 0 0	0 0 0 0	0 Tr 30 40
	M/2500	0 10 20 30	5 10 10 15	0 Tr 5 5	10 20 30 40
	M/5000	Tr 10 25 40	Tr 5 10 15	.....	.....
Tannic Acid	Control		10 15 15 15	25 35 35 45	
	1:500	0 0 10 40	0 0 0 0	0 0 Tr Tr	
	1:1000	Tr 10 40 60	0 0 0 0	0 0 5 15	
	1:5000	10 20 40 60	0 0 0 0	0 0 25 40	

[a] Indicates no tubes were used with these concentrations. Figures show gas production in 12, 24, 48 and 72 hrs.

CONCLUSIONS

1. Many inorganic astringents and organic astringents (as exemplified by tannic acid) are capable of increasing the germicidal power of the antiseptics used in this study.

2. The effect upon germicidal power resulting from the addition of astringent substances to disinfectants is more pronounced when a gram-negative organism is used than when the test organism is gram-positive.

3. Astringent salts and tannic acid in certain concentrations increase the growth-inhibiting power of the antiseptics against the colon organism.

4. Very small concentrations of the astringents have been found to be capable of increasing the bacteriostatic power of some of the antiseptic agents.

5. The manifestation of a positive increase in inhibiting power seems to be dependent upon the nature and the concentration of both the antiseptic and the astringent. The astringents do not exercise the same degree of effect upon different antiseptics to which they are added.

6. There seem to be maximum dilutions for both antiseptic and astringent beyond which no enhancement of inhibiting effect is manifested by combinations of the substances.

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A Study of the Disinfectant Powers of Certain Astringent Salts and Solutions*†

By Joseph B. Sprowls and Charles F. Poe

Astringent substances are constantly associated in their use with antiseptic agents. The astringent salts are often used in the form of lotions or washes for local application to wounds, or as contractile agents in large ulcers, etc.—conditions in which any disinfectant action which the astringent substance itself may possess would certainly be desirable. Since astringents possess the common property of precipitating protein material, they form a protective layer of coagulated protein about the infected part and thus aid in the repair of the tissue (1). This ability to promote healing of an infected area suggests that these materials may be possessed of antiseptic or disinfectant powers, and probably accounts for the early use of astringent salts, particularly zinc chloride and alum, for purely germicidal purposes.

Though the numerous astringent and so-called "disinfectant" preparations of the past decades have fallen into disuse as antiseptics because of an influx of the superior phenolic disinfectants, the conception that astringent substances possess disinfectant powers has not been entirely abandoned. Evidence to this effect is given both by frequent statements in the literature (2) and by the prevalence of various pharmaceutical preparations (collyria, "antiseptic" douche powders, mouth washes, gargles, etc.) in which astringent salts are included for their presumed germicidal properties. In addition to these miscellaneous preparations, the National Formulary recognizes several astringent solutions to which antiseptic or disinfectant properties are frequently attributed (3).

As is now well known, our conception of the meaning of the word antiseptic has changed in recent years. Whereas early authorities defined antiseptics as substances which have the ability to inhibit bacterial growth without necessarily destroying the organism, constant misuse has brought about the understanding that antiseptics are capable of killing germs (4). This interpretation has been embodied in a statement issued by the Food and Drug Administration (5) which requires that an antiseptic must "destroy bacteria under the conditions of use." The official method of antiseptic testing is designed to determine the maximum dilution of the antiseptic which will kill the test organism in a certain time interval under definite conditions.

A review of the literature in the search for comparative values which could be assigned to the disinfectant powers of the astringent salts and their preparations has revealed that while much information is available concerning their so-called antiseptic action (their ability to inhibit or to limit bacterial growth), very little can be found concerning their germicidal value, especially as determined by the official method of antiseptic testing. The reasons for this are probably the following: (*a*) the relative unimportance of inorganic salts in modern disinfection; (*b*) the fact that older methods of disinfectant testing, which have prevailed in the studies of these salts, were based upon the establishment of bacteriostatic rather than disinfectant powers; (*c*) the further fact that modern methods of antiseptic testing, because of the comparatively short time intervals used, are not particularly adapted to a disinfectant study of the slow-acting inorganic salts.

The purpose of this research was to study the disinfectant powers of a number of astringent salts and official astringent solutions according to modern methods of antiseptic testing.

EXPERIMENTAL

Nearly all inorganic salts show some degree of astringency, the extent of this action being generally proportional to the concentration of the salt, so that most of the inorganic salts might be considered to be

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astringents under certain conditions. However, there are certain salts to which the quality of astringency has been especially attributed, and many of these salts, such as alum and lead subacetate, are widely used because of this quality. Another fact which must be remembered is that astringent, irritant and caustic actions are merely degrees of the same process, and any of these three actions frequently can be obtained from the same salt solution simply by an adjustment of the concentration of the salt.

The classification of salts as astringent or otherwise, then, is an arbitrary one, and the salts chosen for use in this study are merely ones which have been pointed out by authors of various textbooks on pharmacology as having astringent properties. In some instances availability or solubility was the determinative factor. All of the salts used were at least of U. S. P. specifications.

In examining the salts for disinfectant action, stock solutions were made containing definite percentages (usually 10%) by weight of the salt in question. These solutions were then tested for germicidal properties. When a given solution failed to kill the test-organism in this concentration, higher concentrations were tried, and finally saturated solutions were used if the weaker solutions had failed. A study of the activities of molar or partial molar solutions of the salts was also made. In this study molar solutions were tried first, then one-tenth molar or one-hundredth molar stock solutions of the more active salts were prepared. Only freshly prepared solutions were used in conducting the tests.

TABLE I.—MAXIMUM DILUTION OF SALTS REQUIRED TO KILL ORGANISMS IN 10 MIN. BUT NOT IN 5

Salt	Maximum Dilution *E. typhosa*	Maximum Dilution *S. aureus*
$AlCl_3.6H_2O$	1:3	....[a]
$Al_2(SO_4)_3.18H_2O$	....	....
Alum (Potassium)	....	....
$CoCl_2.6H_2O$	1:20	1:6
$Co(NO_3)_2.6H_2O$	1:5	1:5
$CuCl_2.2H_2O$	1:300	1:7
$Cu(NO_3)_2.3H_2O$	1:50	1:6
$CuSO_4$	1:40	....
$FeCl_3.6H_2O$	1:200	1:10
$Fe_2(SO_4)_3$	1:500	1:30
$Pb(C_2H_3O_2)_2.3H_2O$	1:3	....
$Pb(NO_3)_2$	1:3	....
$HgCl_2$	1:25,000	1:5000
$NiCl_2.6H_2O$	1:5	1:3
$Ni(NO_3)_2.6H_2O$	1:3	1:3
K Antimonyl Tartrate	1:20	....
$AgNO_3$	1:1000	1:700
$SnCl_2.2H_2O$	1:350	1:70
$Zn(C_2H_3O_2)_2.2H_2O$	1:3	....
$ZnCl_2$	1:8	....
ZnI_2	1:30	1:10
Zn Phenolsulfonate	....	....
$ZnSO_4.7H_2O$	1:3	....
Phenol	1:90	1:60

[a] Indicates that a saturated solution of the salt did not kill.

The Food and Drug Administration method of antiseptic testing was used (5). The test organisms were *Eberthella typhosa* and *Staphylococcus aureus*. The *p*H readings were taken by means of the Hellige *p*H meter.

Maximum dilutions of the salts which killed in 10 min. but not in 5, as determined by using the organisms *E. typhosa* and *S. aureus*, are given in Table I.

The activities as listed in Table I are expressed as dilutions rather than as phenol coefficients, so that one may readily observe the high concentrations of the salts which in some instances must be present in order to destroy the bacteria in the given time interval. A consideration of the data presented in this table reveals the fact that neither of the organisms is particularly susceptible to the action of many of the salts. In every instance *S. aureus* shows a greater resistance than *E. typhosa*. There does not seem to be any constant ratio between the killing dilutions as established against the two organisms when different salts were used. Cooper and Forstner (6) have pointed out that *S. aureus* is very resistant to the action of germicides, such as nitroso compounds and salts of the heavy metals which owe their efficacy to definite chemical action upon the protoplasmic constituent of the organism.

There is a general similarity between the series of bactericidal activity as recorded in Table I and the series of astringency as agreed upon by most authors (7).

Table II presents the killing dilutions as determined for one-molar solutions of the salts. The stock solutions were not always one-molar, but the maximum dilution is calculated in each instance as it would be for a solution of this concentration. A statement of the molar concentration of the stock solution used in each experiment, and of its *p*H, is given.

A study was made of the germicidal power of some of the acids which correspond to each salt used. The results of this investigation are presented in Table III.

It is apparent that the hydrogen-ion concentration is an important factor in disinfection by the mineral acids. This conclusion has been expressed by Winslow and Lochridge (8) and by Norton and Hsu (9). The action of acetic acid is rather unusual in that it is effective at a much lower degree of acidity than are the other acids. Winslow and Lockridge have advanced the hypothesis that in disinfection by this acid the undissociated molecules play an important part.

Since the salts studied hydrolyze in solution to form a hydrated oxide and the free acid, it might be thought that the acids produced by hydrolysis are more important in disinfection by these substances than are the salts themselves. Although it cannot be said that this is never true, it certainly is not a general rule. The copper salts are very active against *E. typhosa* at a *p*H of 3, whereas the in-

TABLE II.—DILUTIONS OF MOLAR SOLUTIONS OF THE SALTS WHICH KILLED IN 10 MIN. BUT NOT IN 5

Chemical Formula of the Salt	Molar Concentration of Stock Solution	*p*H of Stock Solution	Maximum Dilution[a]	
			E. typhosa	*S. aureus*
$AlCl_3.6H_2O$	1.0	2.40	....[b]	....
$Al_2(SO_4)_3.12H_2O$	0.1	3.31	....	....
$KAl(SO_4)_2.12H_2O$	0.1	3.30	....	....
$Cu(NO_3)_2.3H_2O$	1.0	2.90	1:8	1:1
$CuCl_2.2H_2O$	0.1	3.60	1:35	1:10
$CuSO_4$ (anhyd.)	0.1	4.05	1:10	....
$CuSO_4$ (anhyd.)	1.0	3.02		....
$Co(NO_3)_2.6H_2O$	1.0	2.15	1:1	1:1
$CoCl_2.6H_2O$	1.0	3.30	1:3	1:1
$FeSO_4.7H_2O$	0.1	4.20	....	....
$FeCl_3.6H_2O$	0.1	1.55	1:25	
$FeCl_3.6H_2O$	1.0	0.53	....	1:4
$Fe_2(SO_4)_3$ (anhyd.)	0.1	1.20	1:150	1:10
$Pb(C_2H_3O_2)_2.3H_2O$	1.0	5.50	....	....
$Pb(NO_3)_2$	1.0	3.40	....	....
$HgCl_2$	0.001	5.10	1:4000	1:1000
$NiCl_2.6H_2O$	1.0	4.00	....	....
$Ni(NO_3)_2.6H_2O$	1.0	3.20	....	....
K Antimonyl Tartrate	0.1	4.50	....	....
$AgNO_3$	0.1	4.60		....
$AgNO_3$	0.01	5.39	1:140	
$SnCl_2.2H_2O$	0.1	1.80	1:70	1:14
$Zn(C_2H_3O_2)_2.3H_2O$	1.0	5.95	....	....
$ZnCl_2$ (anhyd.)	1.0	5.35	1:1	....
ZnI_2	1.0	2.47	1:6	1:2
Zn Phenolsulfonate	0.1	6.40	....	....
$ZnSO_4.7H_2O$	1.0	4.90	....	....

[a] Calculated in each instance as the dilution of a one-molar solution of the salt.
[b] Indicates that the stock solution used did not kill in 10 min. under the conditions of the test.

TABLE III.—KILLING POWER OF SOME ACIDS

Chemical Formula of the Acid	Approximate Normality of Maximum Dilution[a]	*p*H of Maximum Dilution[a]
	E. typhosa	
HNO_3	0.010	2.05
HCl	0.014	2.00
H_2SO_4	0.020	1.90
$HC_2H_3O_2$	0.200	2.85
	S. aureus	
HNO_3	0.10	1.00
HCl	0.20	0.95
H_2SO_4	0.33	0.90
$HC_2H_3O_2$	1.25	2.50

[a] By maximum dilution is meant the greatest dilution which kills in 10 min. but not in 5 min.

organic acids are only active in this time interval at a *p*H of 2, a hydrogen ion concentration 10 times as great. This instance is but one of the many examples that are apparent.

A study of the germicidal value of the official astringent solutions revealed that only 3 of these are capable of destroying *S. aureus* under the conditions of the F. D. A. test in 10 min. These 3 preparations were Solution of Ferric Chloride, Solution of Ferric Sulfate and Solution of Ferric Subsulfate. Solution of Aluminum Acetate and Solution of Aluminum Chloride were found incapable of destroying *E. typhosa* in 10 min. Solutions of Ferric Chloride, Ferric Sulfate, Aluminum Subacetate, Ferric Subsulfate, Lead Subacetate, Zinc Chloride and Diluted Solution of Lead Subacetate were found to be capable of killing this organism. Innumerable allusions to the antiseptic property of these solutions may be found in the literature. In view of the findings, it would seem that some changes are in order for the literature regarding the value of these preparations.

CONCLUSIONS

1 The disinfectant powers of 24 astringent salts and of 11 official astringent solutions have been studied by the F. D. A. method, with *Eberthella typhosa* and *Staphylococcus aureus* as the test organisms.

2. With the exception of mercuric chloride and silver nitrate, none of the salts studied were found to be particularly effective against the test organisms.

3. The typhoid organism was found to be much more susceptible to the action of the salts than was *S. aureus*. Salts of copper, iron and tin were found to be fairly effective against *E. typhosa* but practically inactive against the staphylococci.

4. A study of the official astringent solutions has shown that much of the literature concerning their disinfectant powers is in need of revision.

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Fluorine Substituted Aromatic Acids*

By G. P. Hager† and E. B. Starkey

Attempts have been made during recent years to determine the effect of fluorine on the pharmacological and antibacterial properties of organic compounds by studying the fluorine derivatives or analogues of compounds of known physiological importance. The preparation of a series of monofluorine substituted aromatic acids and the study of their anti-bacterial properties and toxicity have been undertaken in this work. The unsubstituted acids as well as the parabromine substitution derivatives have been included for comparison. Such an investigation of these acids was considered especially appropriate since their use in preservatives and medicinals depends on the properties which have been critically examined.

The halogen substituted acids with the exception of *p*-bromophenylacetic acid were prepared from the corresponding halogen substituted toluenes. The fluorotoluenes were prepared by the procedure originally described by Balz and Schiemann (1) which involves the heat decomposition of the corresponding tolyldiazonium borofluorides. *p*-Bromotoluene was prepared by the heat decomposition of the double salt of *p*-tolyldiazonium sulfate and mercuric bromide by the method of Schwecten (2).

Bromination of the halogen substituted toluenes and hydrolysis of the resulting mixture served as a convenient method for the preparation of the halogen substituted benzyl halides, benzaldehydes and benzoic acids. The benzyl halides were then used in the preparation of the halogen substituted phenylacetic acids by conversion into the nitrile and acid hydrolysis. The halogen substituted benzaldehydes were converted into the corresponding cinnamic acids by a Perkin or Knoevenagel reaction, and into the corresponding mandelic acids by hydrolysis of the mandelonitriles prepared from them.

A comparison was made of the toxicities of *p*-fluoro-, *p*-chloro-, *p*-bromo-, *p*-iodo- and unsubstituted benzoic acids. Aqueous solutions of the sodium salts were injected at a rate of 1 cc. per minute into the saphenous vein of white rats weighing from 90 to 150 Gm. Five rats were used for each dose. The LD_{50}'s were calculated from the resulting percentage mortalities by the method described by Kärber (3). The standard deviations of these values were calculated by the approximate method described by Irwin and Cheeseman (4). This method involves a fitting of the observed proportional responses to a straight line and in this way estimating the true proportional responses before applying the usual formula. The weighted means of several determinations and their standard errors were com-

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TABLE I.—TOXICITY OF HALOGEN SUBSTITUTED BENZOIC ACIDS

Compound	Date of Expt.	LD_{50} ± S.D. (P = 0.66)	Mean LD_{50} ± S.E. (P = 0.66)
Benzoic acid	5/8/41	1.686 ± 0.105	
Benzoic acid	5/23/41	1.855 ± 0.076	1.714 ± 0.037
Benzoic acid	6/25/41	1.630 ± 0.062	
p-Fluorobenzoic acid	5/8/41	1.687 ± 0.109	
p-Fluorobenzoic acid	5/23/41	1.982 ± 0.137	1.542 ± 0.107
p-Fluorobenzoic acid	6/25/41	1.434 ± 0.055	
p-Chlorobenzoic acid	6/25/41	0.838 ± 0.033	
p-Bromobenzoic acid	5/23/41	0.812 ± 0.043	0.812 ± 0.042
p-Bromobenzoic acid	6/25/41	0.812 ± 0.041	
p-Iodobenzoic acid	6/25/41	0.786 ± 0.037	

puted in the usual manner. The results of these experiments appear in Table I.

By the usual method it can be shown that the toxicities of benzoic acid and its *p*-fluoro-substitution derivative are not significantly different; that the toxicities of *p*-chloro-, *p*-bromo- and *p*-iodobenzoic acids are not significantly different and finally that the toxicities of the latter three acids are greater than the toxicities of the former two acids. A regression line of the toxicity of benzoic acid is steep, indicating a uniformity in the response of white rats so treated.

The bacteriostatic properties of the acids were determined by incubating a 24-hr. broth culture of *Eberthella typhi* and *Staphylococcus aureus* in nutrient broth at *p*H 5 in the presence of the acids in concentrations varying from 0.01 to 0.0005 *N* (from about 1:800 to 1:10,000). The maximum effective dilutions observed at the end of 24 hrs. and one week were practically the same. The latter values appear in Table II. A nuclear halogen increased the bacteriostatic activity of all the acids on *S. aureus*, the bromine substituted acids being considerably more active than the fluorine substituted acids. With the exception of the halogen substituted phenylacetic acids and *o*- and *m*-fluorobenzoic acids, a nuclear halogen increased the bacteriostatic activity of the acids against *E. typhi*. The bromine substituted acids were more active on the latter organism than the fluorine substituted acids with the exception of *p*-fluorophenylacetic acid (more active than *p*-bromophenylacetic acid) and the fluorocinnamic acids (more active than *p*-bromocinnamic acid).

With the exception of the fluoromandelic acids, the halogen substituted acids were more active against *S. aureus* than they were against *E. typhi*. This selective activity was particularly marked in the case of the bromine substituted acids, and is to be ascribed to a "quasi-specific" effect previously demonstrated by Bittenbender and Degering (5) using *p*-bromophenylacetic acid. This phenomenon consists of a specific antibacterial activity against *S. aureus*, and was first described in investigations on halogen substituted alkylphenols (6). This effect was so pronounced in the case of *p*-bromocinnamic acid that growth of *E. typhi* was positive in all dilutions (approximately 1:800 to 1:10,000), while that of *S. aureus* was negative in these same dilutions. This substance has a possible use in the preservation of blood cultures in the diagnosis of *E. typhi* infections.

It has been suggested that antibacterial activity in a series of related compounds can be correlated with the relative solubilities of the compounds in oil and water (7). The ratios of the concentrations of the acids in cottonseed oil and water after shaking solutions of the acids in mixtures of equal parts of the oil and water for 18 hrs., were determined. If the acids were divided into two groups on the basis of their antibacterial activities and into 2 groups on the basis of the ratios of their solubilities in oil to their solubilities in water, then 80% of the acids in the active group also appeared in the group which had high values for the ratio. The values of these ratios (concentration in oil/concentration in water) appear in Table II.

Analyses of new compounds were carried out by a semi-micro combustion method using 20-mg. samples.

TABLE II.—SUMMARY OF BACTERIOSTATIC TESTS AND RATIOS OF SOLUBILITIES IN OIL AND WATER

Compound	Maximum Effective Dilution 1 Week[a] *S. aureus*	*E. typhi*	Distribution Ratio[b]
Benzoic acid	1:4367	1:3279	5.37
p-Fluorobenzoic acid	1:3802	1:2857	6.35
o-Fluorobenzoic acid	1:2857	1:1905	2.06
m-Fluorobenzoic acid	1:5714	1:3802	6.61
p-Bromobenzoic acid	−	1:4980	14.34
Phenylacetic acid	1:2941	1:2941	2.25
p-Fluorophenylacetic acid	1:3436	1:2577	2.41
o-Fluorophenylacetic acid	1:3436	1:1721	1.88
p-Bromophenylacetic acid	−	1:2475	15.08
Cinnamic acid	1:7752	1:3597	3.36
p-Fluorocinnamic acid	−	1:4808	4.11
o-Fluorocinnamic acid	1:8000	1:4808	9.20
m-Fluorocinnamic acid	−	1:4132	17.00
p-Bromocinnamic acid	−	+	7.00
Mandelic acid	1:877	+	0.22
p-Fluoromandelic acid	1:940	1:1176	0.54
o-Fluoromandelic acid	+	1:784	0.17
m-Fluoromandelic acid	1:940	1:940	0.20
p-Bromomandelic acid	1:2252	1:1733	1.20

[a] A minus sign indicates that no growth occurred in any dilution, and a plus sign indicates that growth occurred in all dilutions.

[b] A fractional ratio indicates that the compound was more soluble in water than in oil.

EXPERIMENTAL

Preparation of Tolyldiazonium Borofluorides.—Three methods were employed in the preparation of the diazonium borofluorides.

(*a*) To 200 cc. of hydrochloric acid (sp. gr. 1.19) contained in a 2-L. beaker and stirred with a mechanical stirrer, was added 107 Gm. (1 mol) of the toluidine. The solution of the toluidine hydrochloride was chilled to 5–10° C. and some of the salt crystallized. To the well-stirred suspension was added dropwise a solution of 69 Gm. (1 mol) of sodium nitrite, keeping the temperature at 5–10° C. If a positive test for excess nitrous acid was not obtained after completion of the addition of the sodium nitrite solution, sufficient additional 40% sodium nitrite solution was added to provide a slight excess of nitrous acid. To the cold solution of the diazonium chloride was added a cold filtered solution of 272 Gm. (2 mols) of sodium fluoborate in sufficient water, and the mixture was stirred for 15 min. The solid was collected on a fritted glass funnel, washed twice with alcohol and ether successively and dried over phosphorus pentoxide in a vacuum desiccator.—*p*-Tolyldiazonium borofluoride, 64%, dec. 107° C.

(*b*) Better yields were obtained when the hydrochloric acid and sodium fluoborate in procedure (*a*) were replaced by 525 cc. of 42% fluoboric acid (2.5 mols).—*p*-Tolyldiazonium borofluoride, 75%, dec. 107° C.; *o*-tolyldiazonium borofluoride, quantitative yield, dec. 106° C.

(*c*) The method which was found to be most convenient and productive of the best yields in some cases consisted in suspending 143 Gm. (1 mol) of the purified toluidine hydrochloride in 315 cc. (1.5 mols) of 42% fluoboric acid. The sodium nitrite solution was added and the diazonium borofluoride isolated as in procedure (*a*).—*p*-Tolyldiazonium borofluoride, 85%, dec. 107° C.; *o*-tolyldiazonium borofluoride, 82%, dec. 106° C.; *m*-tolyldiazonium borofluoride, 85%, dec. 108° C.

Preparation of the Fluorotoluenes.—The preparation of fluorotoluenes by thermal decomposition of tolyldiazonium borofluorides was carried out by a method similar to that described by Balz and Schiemann (1).

A 100–150-Gm. portion of the thoroughly dried diazonium borofluoride was placed in a 500-cc. distilling flask with a side arm bent at such an angle that the flask could be clamped in a horizontal position and so tilted that the fluorotoluene condensing in the neck would flow into the side arm. The flask was stoppered and connected with a condenser fitted with an especially prepared adapter of large bore which reached almost to the bottom of a 1-L. Erhlenmeyer flask, used as a receiver. The receiver was chilled by a bath of running water and connected with another 1-L. Erhlenmeyer flask fitted with a glass tube of large bore which delivered the gases liberated in the reaction into a hood.

The charge in the distilling flask was heated carefully with a small flame, beginning at the neck of the flask and working around the edges. The rate of heating was governed by the rate at which nitrogen and boron trifluoride were liberated. After the decomposition was complete, the residue was heated more strongly in order to distill off the fluorotoluene. The distillate was shaken with an equal volume of saturated solution of sodium carbonate and distilled with steam. The fluorotoluene was separated from the distillate and dried over anhydrous sodium sulfate.—*p*-Fluorotoluene, 77%, b. $p_{.787}$ 110° C.; *o*-fluorotoluene, 67%, b. $p_{.763}$ 114° C.; *m*-fluorotoluene, 60%, b. $p_{.766}$ 123° C.

Preparation of p-Bromotoluene.—In a solution of 22 cc. of concentrated sulfuric acid in 100 cc. of water contained in a 1-L. beaker was dissolved 26.8 Gm. (0.25 mol) of *p*-toluidine. The mixture was cooled to 0–5° C. by an ice bath. While stirring with a mechanical stirrer, a solution of 17.3 Gm. (0.25 mol) of sodium nitrite in 37 cc. of water was added dropwise to the suspension of the *p*-toluidine salt, keeping the temperature at 0–5° C. Additional 40% solution of sodium nitrite was added if necessary to produce a slight excess of nitrous acid.

During the diazotization, a solution of 190 Gm. (0.5 mol plus excess) of mercuric bromide and 66 Gm. (0.5 mol plus excess) of potassium bromide in 200 cc. of water was prepared. To the solution of the diazonium salt was added slowly the solution of mercuric bromide and stirring was continued until a uniform suspension of a grayish white solid resulted. The solid was collected on a sintered glass funnel with suction, washed with alcohol and ether, and dried over phosphorus pentoxide in a vacuum desic-

cator for at least 2 hrs. The resulting grayish white solid was found to be quite stable to shock, trituration and light. A sample was kept for weeks without any change.

The dry double salt was mixed by trituration with double its weight of potassium bromide. A sufficient quantity of the mixture was placed in a 500-cc. distilling flask to fill one-half of the bulb. The side arm of the flask was so bent and of sufficient length to reach the center of the bulb of another 500-cc. distilling flask when the decomposition flask was clamped in a horizontal position. The receiver was cooled by a stream of running water. The side arm of the receiver was connected to a water pump. With a slow stream of water running through the pump to catch any escaping vapors of mercuric salts, the flask was heated cautiously, beginning at the neck of the flask and working around the edges of the charge until decomposition was complete. The water was fully turned on and the *p*-bromotoluene distilled under reduced pressure from the reaction mixture.—*p*-Bromotoluene, 56% (overall), b. $p._{760}$ 185° C.

Preparation of Fluorobenzyl Bromides.—In a 1-L., round-bottom flask illuminated by a 500-watt projection bulb and a 200-watt clear bulb in suitable reflectors and heated by an oil bath was placed 110 Gm. (1 mol) of the fluorotoluene. The flask was fitted with a thermometer, a dropping funnel whose stem reached nearly to the bottom, a mechanical stirrer and a reflux condenser. The latter was fitted to a glass tube arranged to deliver the hydrogen bromide liberated close to the surface of 2 L. of water contained in a beaker. With vigorous stirring there was added in a fine stream 160 Gm. (1 mol) of bromine at a bath temperature of 110–120° C., the rate of addition being governed by the color of the contents of the flask. The time required varied from $^1/_2$ to 1 hr.

To the highly lacrymatory brominated oil was added an equal volume of formic acid (sp. gr. 1.2), and the mixture was refluxed for 6 hrs. The organic layer was taken up in ether and the aqueous layer was extracted with ether. The fluorobenzoic acid was extracted from the combined ether solution with 10% sodium hydroxide solution and purified in the usual manner. The ether solution was then stirred with saturated sodium bisulfite solution until no more bisulfite addition product formed. The fluorobenzaldehyde was obtained by hydrolysis of the bisulfite addition product with 6 *N* sulfuric acid. Hydrogen bromide was passed into the remaining ether solution in order to convert any fluorobenzyl alcohol into the bromide. After washing the ether solution of the fluorobenzyl bromide with 10% sodium carbonate solution and drying over anhydrous sodium sulfate, the crude fluorobenzyl bromide was obtained by distilling off the ether. The crude material was either used directly in the preparation of the fluorobenzyl cyanide or was purified by distillation under reduced pressure.

By varying the temperature and the amount of bromine added, the proportion of acid, aldehyde and benzyl halide could be varied. A very low yield of *o*-fluorobenzaldehyde was obtained since it was found impossible to prepare a solid bisulfite addition product in the ether solution. It was later observed that pure *o*-fluorobenzaldehyde does form an insoluble bisulfite addition product.

Using 1 mol of bromine for each mol of *p*-fluorotoluene, there was obtained: *p*-fluorobenzyl bromide, 42%, b. $p._{10}$ 83–93° C., and *p*-fluorobenzaldehyde, 6.2%.

Using 1.5 mols of bromine for each mol of *o*-fluoro toluene, there was obtained: *o*-fluorobenzyl bromide, 18%, b. $p._{10}$ 75° C., and *o*-fluorobenzaldehyde, 2.5%.

Using 2 mols of bromine for each mol of *m*-fluorotoluene, there was obtained: *m*-fluorobenzyl bromide, 16.5%, b. $p._{18}$ 146–54° C.; *m*-fluorobenzaldehyde bisulfite addition product, 41.5%; and *m*-fluorobenzoic acid, 5%.

Using 1 mol of bromine for each mol of *m*-fluorotoluene, there was obtained: *m*-fluorobenzyl bromide, 63%; *m*-fluorobenzaldehyde bisulfite addition product, 3%; and unchanged *m*-fluorotoluene, 25%.

Preparation of Fluorobenzyl Cyanides.—The fluorobenzyl cyanides were prepared by the action of potassium cyanide on an alcoholic solution of the fluorobenzyl bromide by a method described by Adams and Thal (8).—*p*-Fluorobenzyl cyanide, 76%, b. $p._3$ 100–103° C.; *o*-fluorobenzyl cyanide, 70%, b. $p._{18}$ 110–115° C.; *m*-fluorobenzyl cyanide, 72%, b. $p._{768}$ 229° C.

Preparation of Halogen Substituted Benzaldehydes.—The fluorobenzaldehydes and *p*-bromobenzaldehyde were prepared by hydrolysis of the corresponding benzal bromide by the procedure described by Coleman and Honeywell (9) for the preparation of *p*-bromobenzaldehyde. Appreciable quantities of the halogen substituted benzoic acids were obtained by filtration and acidification of the residue of the hydrolysis mixture after the aldehyde had been removed by steam distillation.—*p*-Fluorobenzaldehyde, 75%, b. $p._{773}$ 170° C.; *p*-fluorobenzoic acid, 7%, m. p. 186° C.; *o*-Fluorobenzaldehyde, 92%, b. $p._{760}$ 175° C.; *o*-fluorobenzoic acid, 3.5%, m. p. 124° C.; *m*-Fluorobenzaldehyde, 65%, b. $p._{769}$ 168.5° C.; *m*-fluorobenzoic acid, 4%, m. p. 124.5–125.5° C.; *p*-Bromobenzaldehyde, 26%, m. p. 67° C.; *p*-bromobenzoic acid, 11%, m. p. 255° C.

Preparation of Fluorine Substituted Benzoic Acids.—The fluorine substituted benzoic acids were obtained as by-products in the preparation of fluorobenzyl bromides and fluorobenzaldehydes.

Preparation of Fluorine Substituted Phenylacetic Acids.—To 136 Gm. (1 mol) of the fluorobenzyl cyanide contained in a 1-L., round-bottom flask fitted with a reflux condenser were added 250 cc. of 60% sulfuric acid and 150 cc. of glacial acetic acid. The mixture was refluxed until a sample of the organic layer was almost completely soluble in 10% sodium hydroxide solution. The cooled mixture was poured while stirring into 600 cc. of cold water

and let stand overnight in an ice box. The fluorophenylacetic acid was filtered and purified by recrystallization from water.—*p*-Fluorophenylacetic acid, 61%, m. p. 86° C.; *o*-fluorophenylacetic acid, 30%, m. p. 61.5–62° C.; *m*-fluorophenylacetic acid, not obtained in crystalline form.

Preparation of p-Bromophenylacetic Acid.—In a 2-L., three-neck, round-bottom flask fitted with a dropping funnel, mechanical stirrer and reflux condenser with a gas absorption trap, was placed a solution of 136 Gm. (1 mol) of phenylacetic acid and 10 Gm. of iodine in 600 cc. of glacial acetic acid. Through the dropping funnel was added slowly 168 Gm. (1 mol plus 5% excess) of bromine. Stirring was continued for 3 hrs. The product was poured into 4 L. of water while stirring and let stand until the bromine substituted phenylacetic acid and unchanged phenylacetic acid crystallized. The *p*-bromophenylacetic acid was obtained in pure form by fractional crystallization of the mixture from water, in which it is less soluble than *o*-bromophenylacetic acid and unsubstituted phenylacetic acid.—*p*-Bromophenylacetic acid, 19%, m. p. 118° C.

Preparation of Halogen Substituted Cinnamic Acids.—The halogen substituted cinnamic acids were prepared from the corresponding halogen substituted benzaldehydes by Perkin's synthesis (10) using anhydrous sodium acetate and acetic anhydride, or by a Knoevenagel reaction (11) using malonic acid and ammonium hydroxide in alcohol. The acids were best purified by recrystallization from toluene.—*p*-Fluorocinnamic acid, 56% (Perkin's synthesis); 33% (Knoevenagel reaction), m. p. 209.5° C.; *o*-Fluorocinnamic acid, 60% (Knoevenagel reaction), m. p. 180–181° C.; *m*-fluorocinnamic acid, 49% (Knoevenagel reaction), m. p. 166.5° C.; *p*-bromocinnamic acid, 30% (Knoevenagel reaction), m. p. 260° C.

Preparation of Fluoromandelic Acids.—The fluoromandelonitriles were prepared by the procedure described by Corson (12) for the preparation of mandelonitrile in which the bisulfite addition product reacts with sodium cyanide. The fluoromandelonitrile was transferred to a 2-L., three-neck, round-bottom flask fitted with a reflux condenser, mechanical stirrer and dropping funnel whose stem reached nearly to the bottom of the flask. To this was added 500 cc. of concentrated hydrochloric acid and the mixture was stirred vigorously for 1 to 4 hrs. At the end of this time, a solution of 80 Gm. of sodium nitrite in 300 cc. of water was slowly added through the dropping funnel, keeping the temperature at 20–30° C. After the addition was complete, the mixture was stirred until all the gases were evolved. Unchanged aldehyde was removed from the mixture by extraction with ether or steam distillation. The aqueous solution of the fluoromandelic acid was concentrated to dryness on a steam bath and the residue dried in a vacuum desiccator over anhydrous calcium chloride and soda lime. The thoroughly dried solid was extracted in a Soxhlet extractor with benzene until free of fluoromandelic acid. The fluoromandelic acids were best purified by recrystallization from benzene or toluene.

p-Fluoromandelic acid, 36%, m. p. 133° C. *Anal.*—Calcd. for $C_8H_9FO_3$: C, 56.48%; H, 4.15%. Found: C, 56.33%; H, 4.54%.

o-Fluoromandelic acid, 6%, m. p. 116.5° C. *Anal.*—Calcd. for $C_8H_9FO_3$: C, 56.48%; H, 4.15%. Found: C, 54.95%; H, 4.23%.

m-Fluoromandelic acid, 12%, m. p. 101° C. *Anal.*—Calcd. for $C_8H_9FO_3$: C, 56.48%; H, 4.15% Found: C, 57.09%; H, 4.23%.

Preparation of p-Bromomandelic Acid.—*p*-Bromomandelonitrile was prepared by action of hydrogen cyanide on *p*-bromobenzaldehyde, and was hydrolyzed by concentrated hydrochloric acid by a method described by Jenkins (13) for the preparation of *p*-chloromandelic acid. The *p*-bromomandelic acid was purified by recrystallization from benzene.—*p*-Bromomandelic acid, 30%, m. p. 117.5° C.

SUMMARY

The mono-fluorine substitution derivatives of benzoic, phenylacetic, cinnamic and mandelic acids have been prepared and their antibacterial activity compared with that of the unsubstituted acids and the *p*-bromine analogues.

While a nuclear halogen, especially bromine, increased the bacteriostatic activity of the aromatic acid on *Staphylococcus aureus*, the effect of the activity of the acid on *Eberthella typhi* was quite variable.

It was also found that a fluorine atom in the para position in the benzoic acid molecule has no significant effect on its acute toxicity for white rats, while chlorine, bromine or iodine similarly located almost doubles its toxicity.

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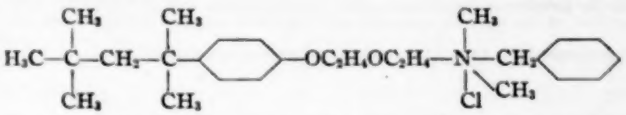
Germicidal Efficacy of Phemerol*

By D. A. Joslyn, Katherine Yaw and A. L. Rawlins

An earlier publication (1) presented the relationship of chemical structure to germicidal activity in a series of quaternary ammonium compounds. One of these, *p-tert*-octylphenoxyethoxyethyldimethylbenzylammonium chloride, was further investigated both in the laboratory and in the clinic, and was recently introduced as a general germicide under the trade name "Phemerol."[1] It has the following structural formula:



It occurs in the form of colorless, odorless crystals containing one molecule of water of crystallization, which soften when heated to 110–120° C., and melt at 165° C. These crystals are extremely soluble in water giving solutions which have a *p*H range of 5 to 6 and which possess a bitter taste. When crystallized from benzene, hexagonal-shaped crystals are formed.

Aqueous solutions of Phemerol are not affected by dilute acid or alkali, but precipitation occurs upon the addition of concentrated solutions of mineral acids. Both aqueous and alcoholic solutions are stable toward light, air and temperatures ranging up to 100° C. They are incompatible with soaps and iodides but do not affect rubber, plastics, fabrics and metals except copper and brass.

Surface tension reducing characteristics of a germicide are of importance because of the resulting increase in spreading and penetrating properties. This property is exhibited to a marked degree by Phemerol, which is a member of the cation-active group of wetting agents. A solution of one part in 1000 parts of water has a surface tension of 36 dynes per cm. at 25° C. (water = 72 dynes per cm. at 25° C.) as determined using the du Nouy Interfacial Tensiometer.

Since the bactericidal properties of Phemerol were reported against *Eberthella typhosa* and *Staphylococcus aureus* (1) a more diverse series of microorganisms has been used in testing the bactericidal action of this compound. All bactericidal assays were carried out by strict adherence to the Food and Drug Administration (F. D. A.) Circular 198 of the U. S. Department of Agriculture (2). Each assay included the "Shippen Technique" (3) which distinguishes bactericidal from bacteriostatic action. According to the above tests the action of Phemerol is bactericidal in nature.

EXPERIMENTAL

The temperatures of 20° C. and 37° C. were employed in making the tests herein reported. These temperatures are in conformity with the F. D. A. Circular 198, which states that for germicides "recommended for use externally the temperature of test should be 20° C., but where such substances are recommended for use in the body cavities...this test should be conducted at 37° C." (2). Furthermore, the same circular states that in such tests, "the information desired is the concentration which kills in 5 min." (2). In performing these tests the

* Received January 6, 1943, from the Research Laboratories, Parke, Davis and Company, Detroit, Mich.

[1] U. S. Trade Mark 305,545.

TABLE I.—GERMICIDAL EFFICACY OF PHEMEROL WHEN TESTED AGAINST TEN DIFFERENT PATHOGENIC BACTERIA

Culture	Dilution Bactericidal in		
	5 Min. at 20° C.	5 Min. at 37° C.	10 Min. at 37° C.
Eberthella typhosa	12,000[a]	20,000	20,000
Escherichia coli	12,000	15,000	15,000
Staphylococcus aureus	25,000	50,000	90,000
Streptococcus (hemolytic)	30,000	60,000	100,000
Pseudomonas aeruginosa	1000 ineffective	3,500	4,500
Diplococcus pneumoniæ	80,000	120,000	180,000
Corynebacterium diphtheriæ	45,000	90,000	120,000
Neisseria gonorrhoeæ	[b]	100,000	100,000
Neisseria intracellularis	[b]	70,000	120,000
Hemophilus pertussis	[b]	20,000	20,000

[a] The figures in the above table, such as 12,000 denote 1 Gm. of chemical diluted with sufficient distilled water to measure 12,000 cc., or it may be expressed 1 in 12,000.
[b] Tests at 20° C. against these organisms were not performed due to their exacting temperature requirements.

TABLE II.—GERMICIDAL EFFICACY OF AQUEOUS AND TINCTURE FORMULAS OF PHEMEROL

Culture	Dilution Bactericidal in					
	5 Min. at 20° C.		5 Min. at 37° C.		10 Min. at 37° C.	
	Aqueous	Tincture	Aqueous	Tincture	Aqueous	Tincture
Eberthella typhosa	9[a]	18	15	25	20	25
Escherichia coli	8	18	15	45	15	50
Staphylococcus aureus	25	30	55	90	70	200
Streptococcus (hemolytic)	40	80	70	100	80	120
Pseudomonas aeruginosa	3	2.5	5	7	5.5	10
Diplococcus pneumoniæ	80	150	120	250	180	350
Corynebacterium diphtheriæ	40	100	80	150	150	200
Neisseria gonorrhoeæ	[b]	[b]	90	100	100	100
Neisseria intracellularis	[b]	[b]	70	120	100	150
Hemophilus pertussis	[b]	[b]	30	25	35	35

[a] The figures in the above table, such as 9, denote the number of times the formula can be diluted and still kill *Eberthella typhosa* under the above conditions. 9 denotes that 1 cc. of the formula is diluted with sufficient distilled water to measure 9 cc., or it may be expressed 1 in 9.
[b] Tests at 20° C. against these organisms were not performed due to their exacting temperature requirements.

preceding stipulations were heeded. However, following a popular precedent, we also carried out tests to show the weakest dilution which kills in 10 min., but not in 5, at 37° C. Since Phemerol, when thus tested, was effective in high dilutions, it is considered to be a potent germicide.

Table I demonstrates the bactericidal action of Phemerol.

This compound has been studied in many different formulas to determine its efficacy in practical forms. Two of the formulas which have been tested are:

Formula No. 1—Phemerol Solution (1:1000 aqueous)

Phemerol.....0.1%
Sodium bicarbonate (reagent).....1.0%
Water, *q. s.*

Formula No. 2—Tincture Phemerol (1:500)

Phemerol.....0.2%
Alcohol.....65.0%
Acetone.....10.0%
Water, *q. s.*

Good germicides are not necessarily fungicidal against the dermatophytes. However, this quaternary ammonium salt has been shown to be effective as a fungicide. Our *in vitro* tests, carried out against seven pathogenic fungi known to be the most common causative agents of the skin diseases generally referred to as ringworm, athlete's foot, etc., were performed in accordance with the method advocated by McCrea (4, 5). In Table III is shown the effectiveness of each of the above formulas against the seven different fungi.

TABLE III.

Fungus	Results in 5 Minutes	
	Phemerol Aqueous 1–1000	Phemerol Tincture 1–500
Actinomyces (Human)	Kills	Kills
Epidermophyton rubrum	Kills	Kills
Microsporum lanosum var. *felineum*	Kills	Kills
Monilia albicans	Kills	Kills
Trichophyton gypseum-asteroides	Kills	Kills
Trichophyton interdigitale	Kills	Kills
Trichophyton violaceum	Kills	Kills

This quaternary ammonium chloride compound, diluted 1–1000 by a quick drying solution, was tested both *in vivo* and *in vitro*. The drying solution, commonly used as a carrying agent for fungicides against the skin fungi, contained 48% alcohol, 2% salicylic

acid and, because of its mild anaesthetic effect, 5% chlorobutanol. In the *in vitro* work this test substance killed all the above listed pathogenic fungi in 5 min. The *in vivo* work was done on guinea-pig lesions caused by *Microsporum lanosum* var. *felineum*. Beneficial results were noted on some animals as early as 3 or 4 days, continuing until the lesions were healed, while the controls were still infected. The results obtained with this substance compare favorably with those obtained with other markedly effective fungicides, and this quaternary ammonium compound has the advantage of being colorless and water-soluble.

CONCLUSIONS

1. Phemerol (*p-tert*-octylphenoxyethoxyethyldimethylbenzylammonium chloride monohydrate) is effectively bactericidal when examined against 10 pathogenic test-microorganisms, and its germicidal action is not reduced when incorporated as the active ingredient in either aqueous or tincture formula.

2. This quaternary ammonium compound is effective as a fungicide in tests *in vitro* and shows promise of value for use against actual lesions caused by fungi.

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Polymorphism of Riboflavin*

By John A. Means, Thomas C. Grenfell and F. Howard Hedger†

In 1935 P. Karrer and others (1) reported a melting point difference of some 12° C. between synthetic and natural riboflavin. They did not evolve a clear explanation of the phenomenon and apparently soon dismissed the idea, since in a later paper (2) they placed the melting point definitely at 280° C., the figure accepted for the U. S. P. XII.

R. Kuhn and others (3) found that "lactoflavin preparations" of melting point 271–293° C. were the same in elementary analysis and gave the same tetraacetate. They suggested that traces of impurities might account for the melting-point difference, and later identified the melting point of the synthetic and natural products at 292° C. (4). A mixed melting point showed no depression.

As late as 1939, one of Kuhn's co-workers, H. Rudy, said of the flavins in general (5), "The high and unsharp melting points are little suited for their identification." From his Table 11 (pp. 85–86), it is evident that he still held to a melting range of 292–293° C.

In examining commercial varieties of synthetic riboflavin, we have noted that certain samples melted with decomposition at 280° C., while other samples showed no change at temperatures as high as 286° C. and only melted at 288–291° C. These differences existed even when the samples were run side by side, either by U. S. P. procedure or by use of the Fisher-Johns melting-point apparatus. The fact that no other obvious differences could be observed by the usual method of analysis as recommended by U. S. P. XII recalled our attention to the questions raised by Karrer and Kuhn and prompted an investigation of the melting point, crystalline character and other properties of pure samples of riboflavin.

By varying conditions, riboflavin was subjected to crystallization and the following modifications isolated and examined.

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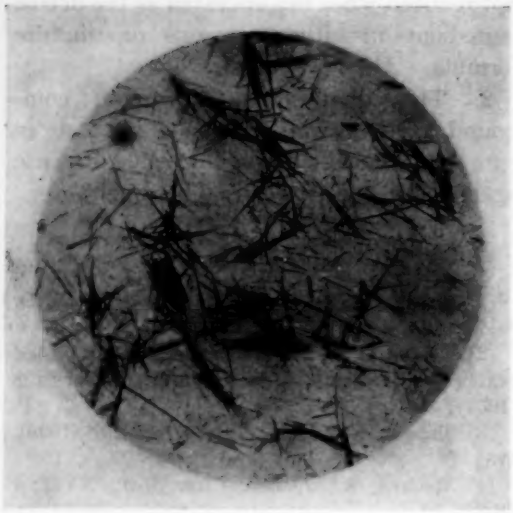

Fig. 1.—Low-melting Needles (×120).

EXPERIMENTAL

I. Low-Melting Needles.—An aqueous solution of riboflavin saturated at 80° C. was cooled to room temperature, filtered and evaporated to one-fifth volume under vacuum maintained by an ordinary water-jet pump. The resulting riboflavin crystals (Fig. 1), when examined microscopically, were found to be small needles, somewhat less silky in character than those which separated from a warm solution. They had a melting point of 281° C. Because of the small size and apparent irregularities of these crystals when dried, it was difficult to observe any distinct optical properties. A photomicrograph of them, however, revealed the structure to be fine crystalline needles.

Fig. 2.—High-melting Needles (×120).

II. High-Melting Needles.—It was found that a conversion to the high-melting form occurred when riboflavin was heated with somewhat less water than required for complete solution. The crystalline product resulting from long boiling under reflux always consisted of high-melting needles having a silky appearance (Fig. 2). This was true irrespective of the type of riboflavin (melting point 280° C., 290° C. or mixture thereof) used in preparation of the solution. The change was not rapid and could easily be followed under the microscope.

Fig. 3.—Low-melting Plates (×120).

III. Low-Melting Plates.—A saturated aqueous solution at 100° C. was cooled to 80° C. and quickly filtered. The filtrate was then allowed to cool slowly over a period of 2–3 hrs. to room temperature. Under these conditions riboflavin separated in the form of crystalline plates, which were filtered off and dried (Fig. 3). The structure of these under the microscope was seen to be entirely different from either of the 2 forms previously described.

Of interest is the fact that a mixed melting point of the 280° and 290° C. materials was found to be 285° C. On many occasions we have observed material crystallizing out which gave definite evidence of 2 separate and distinct crystalline characteristics. This material upon drying gave a melting point between 280° and 290° C.

Commercial synthetic riboflavin of U. S. P. quality was used in the preparation of the 3 types of crystals. The properties so far determined are shown in Table I. Whether the forms belong to different crystal systems will be further investigated.

TABLE I.—PROPERTIES OF RIBOFLAVIN CRYSTALLINE MODIFICATIONS

	Low-Melting Needles	High-Melting Needles	Low-Melting Plates
Melting point[a]	281–282° C.	295–296° C.	281–282° C.
Melting point[b]	277–279° C.	288–291° C.	275–279° C.
Refractive index[c]			
n_α	1.72 ± 0.005	1.72 ± 0.005	1.72 ± 0.005
n_γ	1.96 ± 0.01	1.96 ± 0.01	1.96 ± 0.01
Elementary analysis			
N	14.69%	14.80%	14.73%
C	54.00%	54.15%	54.06%
H	5.30%	5.45%	5.40%
Fluorimetric assay[d]	100% ± 1%	100% ± 1%	100% ± 1%
Specific rotation, $[\alpha]_D^b$	−114° ± 5°	−120° ± 5°	−114° ± 5°
Moisture[b]	1.30%	0.20%	1.31%

[a] Fisher-Johns melting point apparatus.
[b] U. S. P. XII method.
[c] Determination by immersion method.
[d] Pfaltz and Bauer fluorophotometer.

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The Biological Assay of a New Extract of *Convallaria majalis* Leaves Using Pigeons*

By James R. Weeks† and Harald G. O. Holck

During the past few years there has been a renewed interest in therapy using standardized extracts of *Convallaria majalis* leaves, at first in Europe (1, 2, 3, 4), and then in the United States (5, 6). Our preparation[1] was made by a method similar to that described by Straub (7). It consisted of an aqueous extract of the leaves, treated with ferric hydroxide to remove tannins and gums, and then concentrated under reduced pressure until 1 Gm. of the extract was equivalent to 30 Gm. of dried leaves. In an earlier publication (8) we determined the potency of the extract by the 18-hr. frog method, and found it stable, even in water solution at room temperature.

In the present study the potency of the same sample of extract was determined by comparison with U. S. P. XI Reference Tincture of Digitalis by the pigeon emesis method introduced by Hanzlik (9) and extended by Burn (10).

EXPERIMENTAL[2]

One hundred and ten pigeons, 250 to 400 Gm. in weight, were used in each of 2 assays, with a recovery period of 3 weeks between the first and second assays. In the second assay, those pigeons which received convallaria extract in the first now were given digitalis, and *vice versa*. In each assay the pigeons were divided into 2 series of 11 groups consisting of 5 pigeons each. Doses were spaced in equal logarithmic intervals of 0.05 (11.2%).

The pigeons were deprived of food the day preceding the assay. The tincture of digitalis and the convallaria extract were diluted with 0.85% solution of sodium chloride so that 1 cc./Kg. was injected for all doses. Before injection, each pigeon was weighed to the nearest 5 Gm., a few feathers removed from the under side of one wing, and injec-

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† George A. Breon Fellow in Pharmacology, 1941–1942.

[1] This preparation, convara, was furnished by Dr. C. W. Sondern of George A. Breon and Company, Kansas City, Mo.

[2] We are indebted to Miss Beatrice Duis and Mr. James R. Fisher for aid in these experiments.

TABLE I.—FIRST ASSAY: COMPARISON OF CONVALLARIA EXTRACT AND U. S. P. XI REFERENCE TINCTURE OF DIGITALIS

Reference Tincture		Convallaria Extract[a]	
Dose Cc./Kg.	Emesis	Dose Mg./Kg.	Emesis
0.079	0/5	1.12	0/5
0.089	1/5	1.26	1/5
0.100	0/5	1.41	1/5
0.112	2/5	1.58	0/5
0.126	1/5	1.79	1/5
0.141	3/5	1.99	2/5
0.158	3/5	2.24	1/5
0.178	4/5	2.51	2/5
0.199	4/5	2.82	3/5
0.224	5/5	3.16	5/5
0.251	5/5	3.55	5/5
ED_{50}: 0.140 ± 0.012 cc./Kg.		ED_{50}: 2.32 ± 0.14 mg./Kg.	

[a] 0.1 Gm. of convallaria extract is equivalent to 6.03 ± 0.53 U. S. P. XI digitalis units.

TABLE II.—SECOND ASSAY: COMPARISON OF CONVALLARIA EXTRACT AND U. S. P. XI REFERENCE TINCTURE OF DIGITALIS

Reference Tincture		Convallaria Extract[a]	
Dose Cc./Kg.	Emesis	Dose Mg./Kg.	Emesis
0.071	0/5	1.26	0/5
0.079	0/5	1.41	0/5
0.089	1/5	1.58	3/5
0.100	1/5	1.78	3/5
0.112	2/5	1.99	2/5
0.126	2/5	2.24	2/5
0.141	4/5	2.51	4/5
0.158	5/5	2.82	4/5
0.178	4/5	3.16	5/5
0.199	5/5	3.55	4/5
0.224	5/5	3.98	5/5
ED_{50}: 0.125 ± 0.007 cc./Kg.		ED_{50}: 2.02 ± 0.13 mg./Kg.	

[a] 0.1 Gm. of convallaria extract is equivalent to 6.17 ± 0.53 U. S. P. XI digitalis units.

tions made with a 27-gauge needle into the wing vein. Each pigeon was placed in a suitable individual cage for observation. A response was considered negative if no emesis occurred within 45 min. The data are given in Tables I and II.

Using the logarithms of the doses, the log ED_{50} (dose causing emesis in 50% of the birds) was calculated by Kärber's method (11). The standard deviation of the logarithms of the ED_{50}, and the standard error of the ratios of the ED_{50}'s of the reference standard to that of the extract (standard error of the potency of the extract) were calculated by a method by Burn (12) and given in full in our previous study (8). The potencies determined in the 2 assays were averaged and the standard deviation of the average calculated by a formula given by Guilford (13). These assays show that the frog and pigeon methods give different potencies—the values being 10.1 ± 0.5 and 6.1 ± 0.37 U. S. P. XI digitalis units per 0.1 Gm. of convallaria extract, respectively. Although the values for the ED_{50}'s differed in the 2 pigeon assays, the ratio between convallaria and digitalis remained constant, thus showing the necessity of using a reference standard concurrently with each assay.

SUMMARY

The potency of a concentrated, purified, aqueous extract of the leaves of *Convallaria majalis* was found equivalent to 6.1 ± 0.37 U. S. P. XI digitalis units per 0.1 Gm. by the pigeon emesis method. This value is considerably lower than the value (10.1 ± 0.5) previously obtained by the frog method.

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A Semi-Micro Kjeldahl Apparatus*

By Bartlett T. Dewey and Norman F. Witt†

Many variations of Pregl's micro-Kjeldahl apparatus have been designed. In Pregl's original apparatus, the ammonia was steam-distilled from the digestion flask. Heat was applied to prevent condensation of steam during the distillation. Parnas and Wagner (1) designed a widely used apparatus. Condensation of steam was reduced by using a vacuum jacket around the distillation chamber. Schulek and Vastagh (2) criticized the use of rubber stoppers and substituted ground-glass connections in portions of the apparatus where the stoppers come in contact with the distillate. Their apparatus does not employ steam distillation. Kemmerer and Hallett (3) and Fife (4) also eliminated the use of rubber connections and provided a trap to prevent condensation of steam in the distilling flask. Hoppe-Seyler (5), Kirk (6) and Brant and Sievers (7) used a steam jacket around the distilling flask.

These designs possess one or more disadvantages. The apparatus furnished by supply houses, the Parnas-Wagner model, is expensive. A considerable skill in glass blowing is required to construct most designs. The apparatus lacks flexibility. It is easily broken and difficult to repair. The sample must be transferred from the digestion to the distillation flask.

The apparatus shown in Fig. 1 is constructed from stock pieces of glass obtained from the Corning Glass Works, Corning, N. Y. These pieces are listed below with their catalogue numbers:

1. 9185 Tube, connecting, with funnel
2. 8850 Tube, addition
3. 4320 Flask, round bottom
4. 2040 Bulb, connecting, Kjeldahl, Iowa State type
5. 2380 Condensor
6. 6560 Ground Joints, male
7. 6580 Ground Joints, female

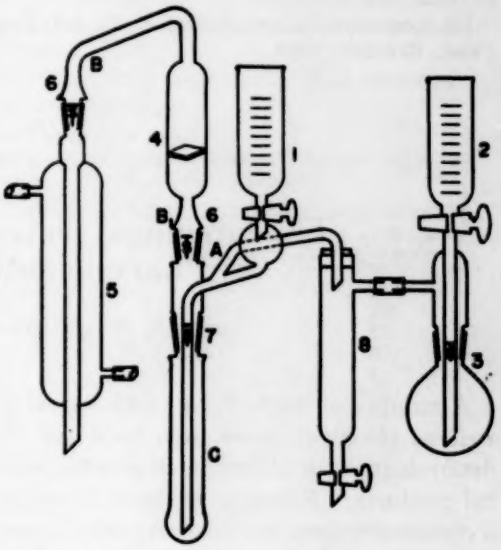

Fig. 1.—Semi-micro Kjeldahl apparatus.

Few manipulations of glass are required: a steam inlet tube must be attached to the addition tube at *A;* standard taper joints must be attached to the connecting bulb at *B* and B_1; the digestion flask, *C*, must be prepared by blowing a bulb on the free end of a standard taper female joint.

The sample is digested and the digestion flask attached by means of its standard taper neck for distillation. Thus the necessity of transferring the sample is eliminated. A trap is used to remove condensation which may form between the steam generator and the distillation flask.

SUMMARY

A semi-micro Kjeldahl apparatus is described. It is readily constructed at about one-half the cost of ready-made apparatus, and broken parts can be easily replaced. It is so constructed that distillation takes place from the same flask in which digestion has been carried out.

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The Chemical Determination of Thiamine in Pharmaceutical Preparations*

By Ernst R. Kirch† and Olaf Bergeim‡

A number of methods, microbiological as well as chemical, have been used for the determination of thiamine in pharmaceutical products. Recently we have described a chemical reagent for thiamine chloride and a procedure which makes possible a quantitative estimation of the vitamin in certain biological materials (1). The method involves the reaction of the thiamine with diazotized ethyl *p*-aminobenzoate. The resulting colored compound is extracted with isoamyl alchohol and the extract compared with a series of standards using a Pulfrich photometer. The method does not require very much time, since the maximum color is produced within 2 min. The color formed is stable for at least 3 months. The test can be applied to solutions of thiamine containing as little as 3 to 6 μg. per 5 cc. of solution.

We have investigated the application of this method to the quantitative estimation of the vitamin in mixtures of pharmaceutical interest. It was found that here also the thiamine did not have to be adsorbed prior to the application of the test, as is true in other methods. The procedure, with some minor modifications, has been found satisfactory for the estimation of thiamine in various products containing this dietary factor.

* Presented to the Scientific Section of the A. PH. A., Denver meeting, 1942.

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EXPERIMENTAL

A. Reagents.—

1. 0.5% solution of the trichloroacetate of ethyl *p*-aminobenzoate (1) in 95% alcohol.
2. 0.5% solution of sodium nitrite in about 85–90% alcohol. (This is best prepared by dissolving 1 Gm. of sodium nitrite in about 10 to 15 cc. of distilled water made up to 200 cc. with 95% alcohol.)
3. 1 *N* sodium hydroxide. (Approximate.)
4. Acetic acid, approximately 10%.
5. Potassium permanganate, 1% solution.
6. Sodium bisulfite, 1% solution, freshly prepared.
7. Isoamyl alcohol.
8. Sodium sulfate, anhydrous.

Solutions 1 and 2 are stable for at least 2 months, if kept in the refrigerator.

A standard thiamine solution can be made to contain 3 mg. of thiamine hydrochloride per L., which can be used for the standard reference curve for thiamine as obtained from the Pulfrich readings.

B. Method.—To an appropriate amount of thiamine solution (3 to 60 micrograms of thiamine per test), add water to bring the volume to about 20 cc. (A 50-cc. Erlenmeyer flask is most convenient to use.) Adjust the *p*H to about 5.5 with the 10% acetic acid. Then add 5 cc. of a diazo solution, prepared by mixing equal quantities of solutions 1 and 2 just prior to the addition. Make the reaction mixture alkaline with 1 *N* sodium hydroxide to *p*H 8 (Nitrazine paper). After 2 min. standing, extract the color with 5 cc. of isoamyl alcohol, which is then separated and dried over anhydrous sodium sulfate. In most cases no difficulty is encountered as far as interferences due to other colors are concerned. Should there be any such color, as in the case of adrenalin or some phenols in higher concentrations, this interference can be eliminated by allowing the mixture to stand for about 12 hrs., after which time only the color produced by the thiamine will be pres-

ent. In some cases a difficulty may be met due to emulsification and this can be overcome by centrifuging the mixture prior to separating the isoamyl alcohol.

C. Application of the Method to Pharmaceutical Products.—

1. Tablets and ampuls of thiamine chloride do not present any difficult problem as far as the analysis of thiamine is concerned. In the case of the tablets, all that is needed after obtaining a uniform and weighed sample is to dissolve an amount equivalent to about 10 micrograms of thiamine per cc.; and in the case of the ampuls, to dilute the sample to about the same concentration. An appropriate amount of these solutions can be used for the assay.

2. Elixirs of thiamine of various manufacture and color were purchased on the market and assayed for their vitamin B_1 content. In no case did any of the colors present in these preparations interfere with the method, since none of them were soluble in the isoamyl alcohol. These preparations were merely diluted to the desired concentration and then tested in the manner described. Table I shows some of the results obtained.

TABLE I.—ANALYSES OF THIAMINE ELIXIRS

Sample	Units of Thiamine per Ounce	
	On the Label	Found
1	2640	2975
2	2200	2400
3	2200	2390
4	2200	2450
5	2200	2510
6	1000	1150
7	1000	910

3. Soft gelatin capsules containing vitamins A, D, B-complex and ascorbic acid were assayed. The other members of the B-complex do not interfere, neither does vitamin D. Vitamin A may impart a red-brown color to the isoamyl alcohol; this can be prevented by shaking a suspension of a particular vitamin mixture with isobutanol or isoamyl alcohol prior to testing for thiamine. Ascorbic acid in concentration of 2 to 5 mg. per 10 cc. of solution will either entirely or partially prevent the formation of the color produced by the reaction of thiamine on the reagent, which might be expected because of the reducing properties of vitamin C (2, 3). The modifications overcoming these interferences were published elsewhere (1). It will suffice to mention that all that is needed is to destroy the ascorbic acid by oxidation with 1% potassium permanganate and decolorizing any excess of the latter with 1% sodium bisulfite before the addition of the diazo solution.

Soft gelatin capsules containing the above-mentioned vitamins have been assayed for thiamine, using these modifications in order to prevent the interferences of vitamins A and C. The capsules were heated with distilled water (50 cc. of water per capsule), and the water-soluble fractions were allowed to dissolve. The liquid was filtered and brought to a definite volume. Aliquot portions were shaken with a small quantity of isobutanol to remove the vitamin A and the oil. The aqueous layer was acidified and the ascorbic acid was oxidized and the resulting mixture assayed for thiamine as described.

Table II gives the results obtained on these types of vitamin preparations.

TABLE II.—DETERMINATIONS OF THIAMINE IN A, B, C, D, G CAPSULES

Sample	Units of Thiamine per Capsule (Label)	Units of Thiamine per Capsule (Found)[a]
1	200	215
2	200	230
3	200	225
4	200	220
5	200	240
6	200	220
7	200	240
8	200	225
9	200	235
10	200	230

[a] These are the averages of two determinations on each sample.

4. Compressed yeast tablets contain a relatively high proportion of their thiamine in the form of the pyrophosphate, cocarboxylase. Since the latter compound does not give the test, it must be hydrolyzed to the thiamine first. This hydrolysis can best be carried out by the use of the enzyme phosphatase which is present in the yeast. The procedure is simple and short. We found that by heating a suspension of fresh yeast in boiling water for about 3 min. the hydrolysis takes place quantitatively. The same method can be applied to these types of tablets. A weighed amount of a representative sample, equivalent to about 1 Gm. of yeast, is suspended in a definite volume of distilled water (20 cc.). This mixture is then heated in a boiling water bath for 3 min. and then filtered. Ten-cubic-centimeter aliquots of this filtrate are used for the test.

DISCUSSION

The method is believed to be specific for thiamine. In testing samples of various material for thiamine, it was found to deviate not more than ±5% from the results obtained by the fermentation method of Schultz, Atkin and Frey (4). Cocarboxylase, unlike the method of the procedure of Melnick and Field (5), does not produce any interfering color. Ascorbic acid as mentioned prevents the formation of color. This can be modified by oxidizing the vitamin C by the use of dilute potassium permanganate solution. A similar modification for the Melnick and Field method was reported by Emmett, *et al.* (6), and by Auerbach (7). The latter worker claims, however, that the oxidation

products of ascorbic acid also interfere with the Melnick and Field method by preventing the color formation. It may be that oxidation carried out under the conditions described by him was incomplete. We did not find any such interference by the oxidation products of the ascorbic acid. Furthermore, potassium permanganate when added to a solution of thiamine at a *p*H 5 to 5.5 will oxidize this compound very slowly (8), while the vitamin C is oxidized at once. We were able to determine thiamine chloride directly in presence of salicylates, benzoates, various amino acids, proteins, sulfanilamide and its derivatives, insulin, dextrose, lactose, fructose, barbiturates and alkaloids. Adrenalin as mentioned does give a color similar to that produced by the thiamine. The color produced by the former is not stable and will fade within 12 hrs., while the color produced by thiamine was found to be stable for at least 2 months. Results of this modification have been published elsewhere (1).

SUMMARY

Thiamine chloride has been determined in various products of pharmaceutical nature by a method that is rapid and simple and does not require any separation of the thiamine by adsorption. The method is based on the fact that thiamine when reacted with diazotized ethyl *p*-aminobenzoate gives a pink to red color. The procedure has been applied successfully to the analysis of material containing as little as 5 μg of thiamine chloride per Gm. of sample.

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A Quantitative Method for the Determination of Mixtures of Barbital and Phenobarbital*

By Bartlett T. Dewey† and Wesley E. Scott‡

Several satisfactory methods for the determination of barbital or phenobarbital are in use. The National Formulary (1) and Methods of Analysis of the Association of Official Agricultural Chemists (2) give directions for an extraction method with gravimetric determination of the material extracted. Extraction methods have also been applied to separate barbital or phenobarbital from basic substances by Payne (3), Warren (4) and Bell (5). A colorimetric method was described by Koppanyi, Murphy and Krop (6). Barbituric acid derivatives give a blue cobalt complex when treated with methanol solutions of cobalt acetate and barium hydroxide. By comparing the color of the unknown with the color produced by solutions of known concentrations, the determination may be applied quantitatively. Barbital and phenobarbital both produce the same color. Schulek and Rozsa (7) titrate a borax solution of barbital with standard silver nitrate solution, using potassium chromate as the indicator. Phenobarbital does not react quantitatively because silver phenobarbital is somewhat soluble under these conditions. In a turbidimetric method proposed by Budde (8) and modified by Kalinowski (9), the barbituric acid derivative is dissolved in an alkaline solution and titrated with standard

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TABLE I.—ANALYSES OF MIXTURES OF BARBITAL AND PHENOBARBITAL

Barbital, %	Nitrogen Calculated, %	Nitrogen Found, %	Barbital Calculated, %	Phenobarbital Calculated, %	Deviation Barbital, %
90	14.91	14.84	87.94	12.06	−2.06
80	14.59	14.53	78.10	21.90	−1.90
70	14.28	14.29	70.48	29.52	+0.48
60	13.96	14.00	61.27	38.73	+1.27
50	13.65	13.65	50.16	49.84	+0.16
40	13.33	13.37	41.27	58.73	+1.27
30	13.02	13.03	30.48	69.52	+0.48
20	12.70	12.68	19.37	80.63	−0.63
10	12.39	12.37	9.52	90.48	−0.48

silver nitrate until a permanent turbidity indicates the end point of the reaction. By a fifth method, barbital or phenobarbital content may be calculated from the per cent nitrogen found by a Kjeldahl analysis.

Barbital and phenobarbital are so similar in chemical behavior and in solubility that the first 4 methods cannot be applied to a quantitative determination of a mixture. The determination of the nitrogen content and calculation of the percentage of each of the constituents offer a possible method of making the analysis. The percentage of each constituent in a mixture containing a common element is given by the formulas:

$$x = \frac{(C - B)100}{A - B}$$

and

$$y = \frac{(A - C)100}{A - B}$$

By substituting the values for A and B, formulas applicable to barbital and phenobarbital are obtained:

$$x = \frac{(N - 12.07)100}{3.15} \qquad \text{(I)}$$

and

$$y = \frac{(15.22 - N)100}{3.15} \qquad \text{(II)}$$

in which x is the per cent of barbital, y is the per cent of phenobarbital and N is the per cent of nitrogen found in the mixture.

The work here reported applies the Kjeldahl analysis to the determination of 9 mixtures of these 2 substances and records the accuracy obtained by these analyses.

EXPERIMENTAL

Mixtures of Mallinckrodt U. S. P. barbital and phenobarbital were prepared containing 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% and 90% of barbital The mixtures were ground in an agate mortar until homogeneous. Four samples of each mixture were analyzed by the Kjeldahl-Gunning method. The percentages of barbital and phenobarbital in the mixtures were calculated by applying the formulas, and the accuracy determined by comparing these results with the known amounts. The results are shown in Table I.

The maximum deviation of the barbital content for any mixture was 2.06%. This resulted from a deviation of 0.07% in the determination of the nitrogen content. These percentages were obtained by including all results. If the one value showing the largest deviation had been discarded, the error would have been reduced one-half. An error as small as 0.01% in the nitrogen content leads to an error of 0.3% or greater in the barbital content. The error in the percentage of barbital for the 36 analyses was 0.05%.

A graph of Equation I was drawn on coördinate paper with the percentages of nitrogen as abscissas and the percentages of barbital as ordinates. The percentages of phenobarbital are also represented, for these values are equal to 100 minus the corresponding percentages of barbital. By locating the point on the graph corresponding to the percentage of nitrogen in a mixture, the percentage of barbital and phenobarbital may be read directly from the graph. The results obtained on the analyses of the nine mixtures are shown in Fig. 1.

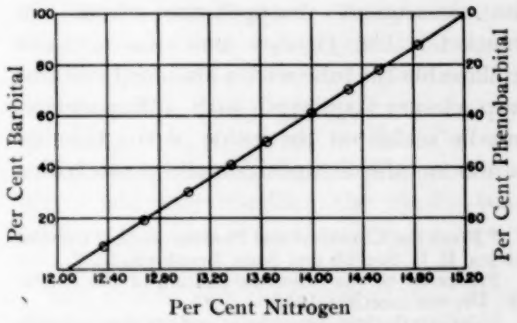

Fig. 1.—Graphical Determination of Barbital and Phenobarbital.

SUMMARY

1. Mixtures of barbital and phenobarbital may be determined by means of a Kjeldahl analysis for the nitrogen content and calculation from equations of the percentages of barbital and phenobarbital.

2. A high degree of accuracy may be obtained only if a large number of analyses are made.

3. Approximate values may be obtained rapidly by a graphical method of solution.

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A Single Dose Emergency Hypodermic Unit*

By J. D. Hulsmann and F. W. Nitardy

Some years ago the War Department indicated that there was need for a self-contained, single dose, hypodermic uinit.[1] During the last war something of this character, known as the Greeley unit, had been used extensively even though it had serious shortcomings, and these may have contributed to its subsequent disappearance from the market. The Greeley unit was a small collapsible tin tube with a standard fold and clip closure, equipped with a hypodermic needle sealed on the inside of the tube by a non-metallic composition and protected on the outside by a glass hood. Solutions packed in it were not stable, and its closure did not provide an hermetic seal. Using the Greeley unit as a starting point, we set about the task of developing something similar but definitely better, as well as the facilities necessary for its manufacture in quantity. The result of this work and some of the problems encountered in its course are herewith briefly described.

The container as finally developed by one of our associates, Mr. Erhard, consists of an ordinary blind end collapsible tube on the neck of which a short nickel-plated ferrule, into which a hypodermic needle is riveted, is held by friction. The needle, and stylet with looped end are made of stainless steel and coated with a germicidal, non-drying film providing permanent freedom from con-

* From the Chemical and Pharmaceutical Laboratories, E. R. Squibb and Sons, Brooklyn, N. Y.

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[1] Various devices, procedures and apparatus herein mentioned have been, or are to be, covered by patent applications or patents (*e. g.*, patent 2,219,301).

tamination. The stylet serves as puncturing instrument for the soft metal diaphragm (blind end) which seals the tube. A plastic hood held on the ferrule by gentle friction protects the needle and stylet and may also simultaneously provide a skin marking pencil through the outer end being dipped in a wax crayon composition. After filling, the tube is sealed by electric welding. The injection solution is rendered bacteriostatic with 0.4% phenol.

To use this collapsible tube syringe, or "Syrette" as it is known in the market, one simply pulls off the plastic hood, then takes hold of the looped end of the stylet with thumb and index finger, pushes it through the blind end of the tube neck, then withdraws it, and the syringe is ready for making the injection.

While this description of the final unit makes it appear that its creation was a rather simple problem, its development and particularly the development of satisfactory means of sterilizing the needle without injuring the tube or creating conditions which make it impossible to seal the tube by welding, as well as perfecting the welding operation, presented perplexing problems. Some of these problems will be briefly discussed here. The preparation of injection solution suitable for packing in such units is described in a separate paper from this laboratory.

A $^3/_8$ x $1^3/_8$-inch soft metal collapsible tube with smooth blind neck is extruded in the usual manner. Pure tin was found to make tubes which were too soft so that some needles would not remain tightly held because the wall of the neck would give too readily. Hence, tin alloyed with a small amount of a harder metal was substituted and this gave the neck wall the required strength. Neck dimensions have to be held to very close tolerances to permit ready and satisfactory attachment of the needle.

The thickness of the sealing diaphragm or blind end of the tube neck has to be held within quite narrow limits in order to permit easy piercing with the stylet.

In drawing the tubes, the use of a special lubricant is held to the absolute minimum, so as to avoid a tube contaminated with lubricant. This is vital not only in order to assure cleanliness, but also reasonably trouble-free welding.

The needle is made from hollow stainless steel wire cut to length. One end is carefully ground to a suitable angle, the other is riveted into a previously made and plated ferrule. The ferrule may be stamped from sheet brass or turned from brass rods. Dimensions accurately held within narrow tolerances are very important. The stylet is made from medium temper stainless steel wire, one end looped for easy grasping, cut to proper length and provided with a protective ring just below the loop or with a small fiber disk so as to protect against pricking the finger when the stylet is used to puncture the sealing diaphragm of the tube.

Both autoclaving and dry heat sterilization of the filled and finished unit proved impractical because of damage done thereby to the protective hood as well as the lithographed label of the tube. However, the needle had to be sterile to prevent infection at the site of injection. We tried autoclaving the unfilled tube and needle and sterilizing the hood by washing in alcohol, also by treatment with chlorine gas. When these methods did not work, dry sterilization of the unfilled tube and needle was tried, but both steam and dry heat sterilization caused trouble with welding the filled tube, apparently because it leads to forming enough tin oxide to result in the appearance of pinholes in the welded seal. Reasoning that dry sterilization in the absence of oxygen should eliminate the difficulty, this was tried next with, however, no greatly different result. The large increase in percentage of leaking syringes found when sterilized in this manner forced us to give up heat sterilization entirely.

The idea was then conceived to place a thin film of a non-drying germicidal agent on the stylet and on the inside and outside of the needle. It would obviate the need for sterilizing the protective hood. This resulted in developing the following method which not only sterilizes the needle but, because the film remains active, preserves it in this conditon.

The needle and that part of the stylet which enters the cannula are coated with a highly mobile volatile solvent solution of an

inert non-volatile oil, capable of leaving a very thin film and containing 0.03% of a mercurial germicide, which has been found free of toxicity, irritation or other objectionable characteristics.

Taking the stylet out of the cannula for the purpose of dipping it and the needle in the sterilizing solution constitutes quite a nuisance, primarily because of the difficulty experienced by the operators in reintroducing the stylet into the needle. Hence, we have developed a valve which is mounted on a small reservoir containing the sterilizing liquid under a few pounds of pressure and as a needle is placed over the valve tip and pressed down slightly, sterilizing solution is forced through the cannula, past the stylet and out at the top. The small droplet exuded from the tip of the needle runs down and because of its particular surface tension characteristics surrounds it completely, thus thoroughly coating it inside and outside, as well as the stylet in a fraction of a second.

Under small production we were able to assemble the unit satisfactorily by hand, but this method which is slow and tedious became quite unsatisfactory for large-scale production. It made necessary the development of a simple device on which 2 tubes and previously sterilized needles are placed and which by means of a single lever movement attaches the needles to the tubes and then holds them in place for placing the hoods over them.

The completely assembled lithographed collapsible tube syringe is then filled through the open end of the tube on a machine equipped with accurate measuring pumps, closing and electric welding facilities. Thus, immediately after the injection solution is filled into the tube, the latter is hermetically sealed, and ready for packing into suitable protective cartons or boxes.

The quantity of solution filled into each tube is 1.5 cc. with a permissible variation of ±0.1 cc. We found it rather difficult to stay within these limits and the fact that we worked with a twin piston unit, filling 2 tubes at a time, did not make things any easier. The filling pumps had to be lubricant-free, to avoid contamination of the solution, hence needed to operate with reasonable clearances. However, we finally solved the problem satisfactorily by maintaining a constant head of solution on the suction side.

The sealing of collapsible tin tubes by electric welding of the open end had been done in our plant on a comparatively small scale for some time and the experience gained in this work led us to believe that it would lend itself to large-scale production on small collapsible tubes and provide an absolutely leakproof hermetic seal.

Notwithstanding our earlier experience with arc welding the flattened end of tin tubes for other products, we found that we encountered 5% to 10% of leakers. It will readily be appreciated that such loss due to faulty welding is unreasonably high and represents serious interference with production, especially on large-scale operations. It also constitutes a recovery problem of no mean proportions.

When we tried autoclaving the assembled units before filling, in order to sterilize the needles, the per cent of leakers went up alarmingly. We attempted to better matters by installing a wiping station, which cleaned first the inside only, then the inside and outside of each tube, but the improvement was so slight that this extra operation seemed hardly worth while.

In order to reduce the number of leakers and to evaluate the various factors contributing to imperfect welding, we ran a great number of experiments using tens of thousands of tubes. We tried tin from different sources, various alloys, tubes drawn with different lubricants, tubes cleaned by an air blast, tubes cleaned with various solvents, tubes cleaned mechanically inside only, as well as on both sides, tubes handled with bare fingers and tubes handled only with gloved fingers, tubes wet steam sterilized, tubes sterilized by dry heat, tubes sterilized and then wiped with white cotton. However, tubes just could not be heat sterilized and then welded satisfactorily. We studied the behavior of filling nozzles of different diameters, of differently shaped discharge tips, and ending at different levels in the tube. In conjunction with the filling machine builder, we experimented with dif-

ferent carbon electrodes and a variety of electric hook-ups. Through all this we finally arrived at a satisfactory procedure.

It is absolutely necessary to keep the tube end not only chemically clean and free from oxide but also perfectly dry. This is no mean task when filling at the rate of 80 per minute. One accidental drop from one of the nozzles wetting the wall of only one tube results usually in 5 to 10 leakers.

The jaws holding the tube end tightly closed during the welding process must of necessity be slightly below the top of the inverted tube. Yet, unless the 2 juxtapositioned tube walls make perfect contact to the very edges, the weld is usually defective. The resulting leaks are often so small that upon squeezing it takes a moment before even a trace of solution shows on the outside.

The less tin is melted, the better the weld. The electrode is usually set to traverse the tube just a few thousandths of an inch below the end. The tin melts before the advancing electrode, forming a bead slightly thicker than the flattened end of the tube. Perfect alignment of the tube ends with regard to height and absolutely true parallel travel of the reciprocating electrode and the stepwise progressing tube holders has to be assured at all times. Through generous cooperation of the machine builder, the Karl Kiefer Machine Company, this has been accomplished and means were provided to automatically compensate for a reasonable amount of play due to normal wear.

After filling and welding, each individual tube is tested by squeezing and if found free from leaks is ready for cartoning or boxing. The inspector has to watch for leaks through the weld and through the needle. The latter occur occasionally when a stylet accidently pierces the blind end of the tube in the process of assembly. Pinholes in the tube, which may be covered by the enamel, do occur sometimes. However, they are detected at the same time.

Recently we have developed and are now using semi-automatic testers, exerting a pressure of approximately 20 lbs. per square inch and in that way we are more certain that no leaky tube goes undetected. While held in this device, the protector of each tube is also turned or lifted slightly to see whether it can be removed easily without loosening the needle or pulling the stylet out of the needle.

Even though leakers have been substantially reduced, there are a few; partly because of imperfect welding, partly for other reasons. When everything is working as it should, leakers do not represent more than 2 to 3% of the units filled.

Recovery of the solution and salvage of tin and needles from rejected units is more of a nuisance than a problem, because quite a few persons are allergic to morphine and similar alkaloidal solutions and suffer from skin irritations if adequate precautions are not observed.

For a time we separated the needles from the rejected or leaky tubes with special pliers which were so formed as not to harm the finish of the ferrule or deform it in any way. This method being rather tedious, we devised a more convenient and yet simple method, in which the tube is blown from the needle with nitrogen gas pressure. This method operates satisfactorily even though the tube end is pierced, so long as a pressure of not less than 600 lbs. is used.

The work was arduous and much of it had to be done under high pressure, but we feel amply rewarded by the knowledge that the "Syrette" proved a most important emergency unit. It rendered invaluable service at Pearl Harbor and elsewhere since, and is highly rated by the medical staff of both our Army and Navy. We are most happy to have had the opportunity to thus assist in rendering a worth-while service to our country's fighting forces. We gratefully acknowledge the assistance rendered by various members of the staff of these laboratories without which we could not have carried this work to successful conclusion.

Book Reviews

Fundamental Organic Chemistry, by ED. F. DEGERING, Department of Chemistry, Purdue University, and collaborators. Planographed by John S. Swift Co., Inc., Cincinnati, Ohio. 1942. x + 485 pp., 15 x 23 cm. Price, $3.00 (also available in paper binding, $2.00).

The arrangement of this textbook of organic chemistry is somewhat different from that which may be considered "usual" among such books. After a general introductory chapter, the authors present a "kaleidoscopic survey" of the field of organic chemistry. This chapter, which covers sixty-eight pages, includes a general discussion of each of the important types of organic compounds, as well as the explanation and application of the concept of relative electronegativity as used to account for the properties of the compounds. While a general survey of the field may very well precede the study of the detailed methods of preparation and the type reactions, much of the material included in this chapter appears to be too advanced for many beginning students of organic chemistry. That the authors recognize that such may be the case is perhaps implicit in the following footnote. "This chapter may be given immediately following Chapter I as a part of a kaleidoscopic review of organic chemistry, or it may be omitted entirely without destroying the logical sequence, or it may be given as a final review. For chemistry majors it should be required. If preferred, appropriate portions of this chapter may be given along with closely related material in subsequent chapters." In a number of the discussions the authors presume the familiarity of the student with such topics as electronic structure, coördination numbers, and bond energies, which he commonly meets subsequent to his course in organic chemistry.

The remainder of the text is developed on the usual classification of organic compounds. The discussion of each group of substances is divided as follows: general discussion, nomenclature, commercial sources, laboratory and special syntheses, physical properties, general reactions, and special reactions. This outline form as well as frequent tabulation of data makes for easy reference. The subject is enlivened by many references to practical applications of the compounds being considered and frequent mention of the composition of familiar trade-marked substances. Excellent collateral reading lists append to many chapters.

Approximately 3 times as much space is devoted to the discussion of the aliphatic compounds as is given to the aromatic compounds. The authors believe this allotment "is justified by the fact that (*a*) the aromatic compounds are considered along with the other series in the kaleidoscopic survey, (*b*) the chemistry of the functional groups is fully covered in the aliphatic series . . . and (*c*) an attempt has been made to correlate the chemistry of the aliphatic and aromatic series throughout the book."

A brief chapter on "Foods and Metabolism" includes sections on enzymes and vitamins, and hormones. Chapters entitled "Miscellaneous Compounds" and "Heterocyclic Compounds," which complete the text, describe a number of natural and synthetic medicinals.—E. L. CATALINE.

The Work Book of Fundamental Organic Chemistry, by ED. F. DEGERING, Department of Chemistry, Purdue University, and collaborators. Barnes and Noble, Inc., New York, N. Y. 1941. vi + 256 pp., 21 x 28 cm. Price, $1.25.

This is a companion of "Fundamental Organic Chemistry" by the same author. It is designed to bring about a "less passive and more active performance on the part of the student." The plan of the Work Book follows that of the textbook "but it should prove to be a valuable teaching aid when used in conjunction with any textbook." Each chapter consists of (*a*) a review summary in outline form of the material covered in the related chapter in the textbook, (*b*) genetic charts, by means of which the important reactions of type compounds are graphically represented, (*c*) nomenclature, pronunciation and formula tables, (*d*) a composite review summary, through which the student may determine his ability to use the information of the text, (*e*) fill-in type of review questions, and (*f*) one or more objective tests.—E. L. CATALINE.

An Outline of Organic Chemistry, by ED. F. DEGERING, Associate Professor of Chemistry, Purdue University. Fourth Edition. Barnes and Noble, Inc., New York, N. Y. 1941. xvi + 386, 13.5 x 21 cm. Price, $1.25.

This outline, as the title indicates, is "not intended as a textbook but as a supplement to any good text" to eliminate most of the necessity for taking lecture notes. The material is presented in the form used in the previous editions and relatively few changes have been made in the content. New chapters have been added on Natural and Synthetic Fibers, Surface Active Agents, and on Scientific Literature. The chapters on Heterocyclic Compounds and on the Mechanism of Organic Reactions have been completely revised and enlarged.—E. L. CATALINE.